THE BIOLOGY AND MANAGEMENT OF AERIAL POPULATIONS OF WOOLLY APPLE APHID, *ERIOSOMA LANIGERUM* (HAUSMANN) (HOMOPTERA: APHIDIDAE).

By

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

ABSTRACT

The basic biology of *Eriosoma lanigerum* (Hausmann) and its natural enemy, *Aphelinus mali* (Haldeman), was investigated in the Western Cape Province of South Africa.

The first instar nymph can be identified by the absence of cornicles and the adult female by the presence of the vulva. Body length and distance between cornicles can be used to distinguish between the 2nd, 3rd and 4th instars.

The development of *E. lanigerum* was negatively influenced by temperatures above 27°C. The net replacement rate (R_0) and intrinsic rate of increase (r_m) peaked at 20°C. The theoretical lower and upper threshold temperatures for development were estimated at 4.48°C and 28.07°C, respectively.

Crawlers of *E. lanigerum* migrated from the roots up into the trees during spring to start new infestations. Colonies became visible from December and maximum colony numbers were reached from the end of summer until autumn, at which time most of the colonies were parasitised by *A. mali.* Colony numbers declined at the end of autumn after high parasitism and the appearance of winged *E. lanigerum*. The aphid overwintered on the tree. Chemical sprays, rain during spring and high temperature influenced population numbers negatively.

E. lanigerum can be monitored by counting the unparasitised colonies in leaf axils of half of each of 25 trees per 2 hectare plot with 5 unparasitised colonies as the economic threshold. Sampling error was high at 40% but increasing the number of trees did not reduce it. Presence-absence sampling, which will reduce the time required for monitoring, did not seriously compromise the reliability of decisions regarding the necessity for intervention.

Chemicals containing nitrogen usually sprayed for bitterpit control stimulated the settling of *E. lanigerum* crawlers on Granny Smith trees, while fruit weevil barriers for the control of *Phlyctinus callosus* Boh. limited crawler movement into the trees but did not prevent colonisation.

All the postembryonic developmental stages of *E. lanigerum* were parasitised by *A. mali*. Complete parasitism of the population was never reached as younger instars sheltered under other aphids and mummified aphids.

Development of *A. mali* was not influenced negatively by high temperatures. The minimum developmental temperature and number of degree days needed for development of the larval stage and emergence of the adult from the mummy were 6.72°C and 172.41°D, and 10.27°C and 109.89°D respectively. Mummies collected during early winter survived long periods of cold storage in postdiapause. The minimum threshold temperature for postdiapause development of *A. mali* was 10.15°C.

Most chemicals tested against *A. mali* adults were highly toxic to the wasp, except endosulfan and two growth regulators, flufenoxuron and fenoxycarb. The mortality of adults exposed to the fungicides tested was low within the first 24 hours. The percentage emergence from the mummies was high for all chemicals tested, but more than 60% of the adults died soon after emergence from mummies treated with chlorpyrifos. Nearly 30% of the adults died soon after they emerged from carbaryl (XLR-Plus) and fenthion treated mummies. The growth regulators, flufenoxuron and fenoxycarb, did not influence fecundity adversely.

OPSOMMING

Die basiese biologie van *Eriosoma lanigerum* (Hausmann) en sy natuurlike vyand, *Aphelinus mali* (Haldeman), in die Weskaap Provinsie van Suid-Afrika isondersoek.

Die eerste instar nimf kan aan die afwesigheid van kornikels en die volwassenes aan die teenwoordigheid van die vulva uitgeken word. Die liggaamslengte en afstand tussen die kornikels kan gebruik word om tussen instar 2, 3 en 4 te onderskei.

Die ontwikkeling van *E. lanigerum* word nadelig deur temperature bo 27°C beïnvloed. Die netto vervangingstempo (R_0) en intrinsieke tempo van toename (r_m) was die hoogste by 20°C. Die teoretiese minimum en maksimum temperatuur drempelwaardes vir ontwikkeling was 4.48°C en 28.07°C onderskeidelik.

In die lente beweeg *E. lanigerum* kruipers op vanaf die wortels tot in die bome om nuwe kolonies te begin. Kolonies is sigbaar vanaf Desember en die hoogste koloniegetalle word aan die einde van die somer tot die herfs bereik, wanneer die meeste van die kolonies dan ook deur *A. mali* geparasiteer word. Teen laat-herfs neem koloniegetalle af as gevolg van hoë parasitisme en die verskyning van gevleuelde *E. lanigerum*. *E. lanigerum* oorwinter op die appelboom. Chemiese behandelings, reën gedurende die lente en hoë temperatuur beïnvloed koloniegetalle nadelig.

E. lanigerum kan deur die aantal ongeparasiteerde kolonies in die blaaroksels van die helfte van 25 bome per 2 hektaar blok te tel, met 5 ongeparasiteerde kolonies as die ekonomiese drempelwaarde, gemonitor word. Die steekproefnemingsfout was hoog (40%), maar kon nie verminder word deur die aantal bome wat gemonitor is te verhoog nie. Steekproefneming, vir aan- of afwesigheid van kolonies, wat monitortyd

sal verminder, het min invloed op die betroubaarheid van besluitnemings oor die noodsaaklikheid van bespuitings gehad.

Stikstofbevattende chemikalieë, wat vir die beheer van bitterpit gespuit word, stimuleer vestiging van *E. lanigerum* kruipers op Granny Smith bome, terwyl snuitkewerversperrings, vir die beheer van *Phlyctinus callosus* Boh., die opwaartse beweging van kruipers in die bome beperk, maar nie kolonievorming van *E. lanigerum* verhoed nie.

Alle postembrioniese ontwikkelingstadiums van *E. lanigerum* is deur *A. mali* geparasiteer. Totale parasitisme is nooit bereik nie, omdat jonger instars onder ander bloedluise en gemummifiseerde bloedluise skuil.

Die ontwikkeling van *A. mali* word nie deur hoë temperature benadeel nie. Die minimum ontwikkelingstemperatuur en graaddae, nodig vir ontwikkeling van die larwale stadium en die verskyning van die volwassene uit die mummie, was 6.72°C met 172.41°D en 10.27°C met 109.89°D, onderskeidelik. Mummies wat vroeg in die winter versamel is, het lang periodes van koelopberging oorleef. Die minimum temperatuur drempelwaarde vir *A. mali* ontwikkeling in postdiapouse was 10.15°C.

Die meeste van die chemikalieë wat getoets is, was hoogs toksies vir die volwasse wesp, behalwe endosulfan en die twee groeireguleerders, flufenoxuron en fenoxycarb. Die mortaliteit van volwassenes wat aan swamdoders blootgestel is, was laag binne die eerste 24 uur na blootstelling. Die persentasie uitkoms vanuit mummies was hoog vir al die chemikalieë wat getoets is, maar met chlorpyrifos het 60% van die volwassenes net na uitkoms doodgegaan. Ongeveer 30% van die volwassenes is dood na verskyning vanuit mummies wat met carbaryl (XLR-Plus) en fenthion gespuit is. Die groeireguleerders, flufenoxuron en fenoxycarb, het nie die vrugbaarheid van die parasiet merkbaar beïnvloed nie.

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TO MY MOTHER

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Woolly apple aphid, *Eriosoma lanigerum* (Hausmann), is a serious pest of apple, *Malus domestica* (Borkh.). It has been distributed on nursery material to almost every apple growing country in the world (Marlatt 1897, Baker 1915, Marcovitch 1934, Schoene & Underhill 1935, Greenslade 1936). The date of entry into South Africa is unknown, but in 1894 it was already regarded as a serious pest throughout the country (Georgala, 1953).

Taxonomic Status

Hausmann first described the woolly apple aphid as *Aphis lanigera* (Baker 1915, Hoyt & Madsen 1960). Baker (1915) established the synonymy of *E. lanigerum* and included a copy of the original description. According to Eastop (1966) *E. lanigerum* belongs to the order Homoptera, superfamily Aphidoidea, Family Pemphigidae and subfamily Pemphiginae. However, Palmer (1952 in Hoyt & Madsen 1960) places *E. lanigerum* in the family Aphidae and subfamily Eriosomatinae. Scholtz & Holm (1985) placed *E. lanigerum* in the order Hemiptera, suborder Homoptera and family Aphididae, with the lower taxa as given by Eastop (1966).

Morphometrics

Reliable criteria for age grading field collected *E. lanigerum* are of critical importance for proper development and interpretations of time specific life table data. Morphometric variation in aphids seems to develop over time (Inayatullah 1985 *in* Asante & Cairns 1995). However, there are differences between the characters used by researchers to distinguish between the instars of *E. lanigerum*. In Australia the first instar had five antennal segments and from the second instar there were six segments (Asante & Cairns 1995). Cornicles were also only visible from the second instar onwards. However, in India the antennae only had six segments from the third instar (Gautam & Verma 1987). They also used the length of the rostrum in relation to the aphid's body to distinguish between the instars. Baker (1915) found that cornicles were present from the first instar, but only from the end of the third instar on did the antenna have six segments, which was more in keeping with Gautam & Verma (1987).

Life cycle and phenology

E. lanigerum was originally a North American insect living on wild apple, mountain ash, hawthorn and American elm, *Ulmus americana* (Baker 1915, Greenslade 1936, Crane *et al.* 1936) . In the eastern United States and Canada it is holocyclic on *U. americana* with a winter egg stage which hatches in the spring to produce a stem mother or fundatrix. They attack an expanding bud of *U. americana*, causing it to form a "rosette" of leaves instead of the normal shoot. Inside the rosette

the fundatrix produces a generation of winged females (spring migrants) which fly to the apple or another summer host and give rise to wingless viviparous females. Several of these generations are produced throughout the summer, and in autumn two forms occur in the same brood: (a) wingless females similar to the summer broods, which remain on the apple all the winter, and (b) winged females (fall migrants) which return to the elm and deposit the sexual males and females. These sexual females produce the winter egg. Thus there are two distinct cycles, one that remains on apple all year round and one that alternates between apple and elm (Baker 1915, Becker 1918, Crane *et al.* 1936).

In other parts of the world the absence of American elm renders the complete cycle impossible and only the apple cycle remains. Sometimes the winged forms are still produced and may give rise to the sexual forms, retaining the holocyclic cycle, but often the egg is not laid or if it is laid it does not hatch (Marlatt 1897, Crane *et al.* 1936, Lal & Singh 1947, Georgala 1953, Hoyt & Madsen 1960, Gautam & Verma 1983), leading to an anholocyclic form.

The apterous female is viviparous and reproduces parthenogenetically on becoming an adult, usually after four molts (Baker 1915, Evenhuis 1958, Gautam & Verma 1987, Asante & Danthanarayana 1990, Asante *et al.* 1991, Asante 1994 b). However, a few individuals may develop into adults after three molts and occasionally after five (Asante *et al.* 1991, Asante 1994 b).

There are differences between countries in the time of appearance of winged females (alates), and their importance in dispersal. In Korea alates appeared only in summer (Nakayama *et al.* 1928 in Schoene & Underhill 1935) while in the Kulu and Kumaun Hills in India the alates appeared from mid-summer with maximum numbers occurring in mid-autumn (Rahman & Khan 1941, Lal & Singh 1947). However, in

Kashmir (also in India) alates appearing from the end of spring until mid-summer produced apterous nymphs and those appearing between the end of summer until mid-autumn produced sexual nymphs (Fotedar & Kapur 1943 in Thakur & Dogra 1980). In Switzerland two kinds of alate females appeared in summer. One produced sexuales and a second, scarcer one, produced young with a long proboscis which fed on apple (Schneider-Orelli & Leuzinger 1926 in Schoene & Underhill 1935). Some individuals produced both types and also a peculiar intermediate form. Kalandadze (1930 in Schoene & Underhill 1935) in Germany reported similar observations. Alates always produced sexuales, and only rarely did young with a long proboscis appear (Kalandadze 1930 in Schoene & Underhill 1935). In California winged woolly apple aphids were unimportant in perpetuating the species as they produced sexual forms which did not mate (Hoyt & Madsen 1960). This confirmed the observations by Schoene & Underhill (1935) who found that in areas where *U. americana* did not occur, the long proboscis form produced by alates were unimportant in the natural spread of woolly apple aphid. Alate females developed mainly in fall and occasionally in summer. Summer alates were scarce and gave birth to the long proboscis form similar to the parthenogenetic type produced by apterous females and by spring alates. Alates appearing in fall were beakless or with rudimentary mouthparts similar to the true sexuales which are commonly produced by fall alates. Rarely a single alate produced some of both forms of progeny. Lal & Singh (1947) as well as Marlatt (1897) believed that alates appeared in summer and that they flew away to start new colonies.

In Australia a few alate virginoparae were present in November and large numbers were found from late January to late April or early May (Asante 1994 b). Alates in November produced apterous virginoparae with fully developed mouthparts.

Alates appearing from late November to late January produced a mixture of sexuales and apterous virginoparae nymphs. The alates that occurred from February to May produced exclusively sexuales while those that appeared in November were capable of spreading the infestation on apples. Alate sexuparae (which produce only abortive sexual morphs) had no significance other than causing a reduction in the aphid populations on apples. This is supported by Hely *et al.* (1982) and Thwaite & Bower (1983) who suggested that the alate females appeared to cause a reduction in the population numbers.

In Holland De Fluiter (1931) also believed that alates were of no significance as they only occurred in low numbers during summer. He found that alates produced young with well-developed mouthparts in mid-summer and sexuales without mouthparts at the end of summer. Evenhuis (1958) also considered the presence of the winged forms of minor importance as they only appeared when *E. lanigerum* reached its highest population density.

During the summer in Southern Africa winged females are produced which are believed to fly off to start new infestations (Georgala 1953, Carnegie 1963, Nel 1983).

There are contradictory reports on migration of *E. lanigerum*. In West Virginia the initial colonization of the aerial portions of apple trees by *E. lanigerum* were from the roots (Brown & Schmitt 1994). Crawlers (the first instar) were found on twigs and buds in the spring (Brown & Schmitt 1994). Aphids had little tendency to wander, but moved down to the roots as temperatures increased with the onset of summer (Lohrenz 1911). However, it seemed as if he was discussing colonies in the laboratory and not under field conditions. In Palestine aerial and root colonies developed independently, with little movement between them (Bodenheimer 1947) while in New Zealand no definite movement to the roots occurred in autumn (Dumbleton & Jeffreys

1938).

A continuous migration between the aerial and subterranean populations has also been reported in Kamaun Hill, India (Lal & Singh 1947) and in California (Madsen & Hoyt 1957). A definite movement down to the ground from the aerial colonies at the end of autumn was recorded by Reppert (1922 in Schoene & Underhill 1935). In addition migration of the virginoparae to the roots has been recorded during the winter in India (Gautam & Verma 1987).

In North America many of the young aphids moved up from the roots in the spring and the young aphids born in early summer migrated to the roots (Baker 1915). However, in South Africa and other countries an upward movement, or an increase in the upward movement, in spring or early summer with a downward movement to the roots from summer until autumn has been reported (Marlatt 1897, Theobald 1920, Schoene & Underhill 1935, Rahman & Khan 1941, Lal & Singh 1947, Hoyt & Madsen 1960, Thakur 1970 in Thakur & Dogra 1980, Nel 1983). A pronounced downward movement usually followed a general aerial infestation in Virginia (Schoene & Underhill 1935). Therefore, downward movement occurred when crawlers were numerous. Except for the seasonal movements at other times of the year (spring) the movements of *E. lanigerum* appeared to be unspecific (Lal & Singh 1947).

The number of crawlers moving up or down can be influenced by different factors. The soil around each tree may be responsible for the difference in the total number of aphids moving on individual trees (Hoyt & Madsen 1960). The type of soil and the extent of cracking affect the degree of infestation on the roots. Sandy soils inhibit infestations and heavy soils which crack favour root infestations (Marcovitch 1934). Rainfall may also affect the number of crawlers that move up from week to

week as the rain seals cracks in the soil or may wash the aphids from the trunks. Rain may also have a cooling effect, which does not favour the production of large numbers of aphids (Hoyt & Madsen 1960). When the cover crop was removed it caused a warming of the soil and the numbers of crawlers moving up increased (Hoyt & Madsen 1960). They also found that the parasitoid, *Aphelinus mali* (Hald.), played a major role in the reduction of the downward movement by reducing the numbers of females present.

Seasonal abundance

E. lanigerum, like other organisms, is a creature of its environment whose population is profoundly influenced by the nature of its host, natural enemies and climatic conditions (Lal & Singh 1947). The apterous female is the form which dominates the aerial parts throughout the winter where it survives in protected sites (Marlatt 1897, Greenslade 1936, Gambrell & Young 1950, Georgala 1953, Evenhuis 1958, Carnegie 1963, Nel 1983, Thakur & Dogra 1980, Asante 1994 b).

Activity starts in spring (Greenslade 1936) or the end of winter (Lal & Singh 1947) when the first 'wool' is noticed on the overwintering aphids. Numbers increase and in the northern hemisphere peak populations are observed from early summer. This is followed by a decline with an increase again towards the end of summer or autumn (Schoene & Underhill 1935, Greenslade 1936, Bodenheimer 1947, Lal & Singh 1947, Evenhuis 1958, Thakur & Dogra 1980, Blommers 1994, Brown & Schmitt 1994). Many explanations for this temporary decline in population numbers during summer have been given. In India the population densities decreased when the

humidity increased and as soon as humidity decreased from the start of autumn aphid numbers increased again (Lal & Singh 1947). In other areas of India colony numbers were governed by rainfall (Thakur & Dogra 1980). Maximum population levels were recorded at the end of autumn when there were scanty rains, while lower numbers of colonies were recorded from summer until autumn when there were heavy downpours. The rate of reproduction slowed down, or even stopped, during severe summer drought in the absence of irrigation and many swarming crawlers failed to settle (Blommers 1994). In Palestine the direct effect of the spring hamsin wind and/or the condition of apple trees may have been the cause of population decline during summer (Bodenheimer 1947). In the Netherlands only a few unhealthy aphids, almost without wool secretion, were present on the trees in midsummer. This was attributed to the physiological condition of the host plant (Evenhuis 1958, 1962). High temperatures (30°C) frequently occur in Tennessee during summer. These were highly unfavourable for *E. lanigerum* (Marcovitch 1934). However, Schoene & Underhill (1935) reported that the aphids could withstand temperatures higher than 30°C.

In Southern Africa colonies increased rapidly during December with peak numbers towards the end of summer. Numbers remained high until colder weather set in (Georgala 1953, Carnegie 1963, Nel 1983). The rapid increase in colony numbers during December coincided with vigorous tree growth (Carnegie 1963). In Australia *E. lanigerum* numbers began to increase in spring when the apple trees started to produce new leaves, flowers and fruit (Asante 1994 a). Population levels continued to increase until peak abundance occurred during late summer to early autumn. The population levels declined from the end of autumn to winter, during which time the apple trees had dropped their leaves and had become dormant.

Effects of temperature

The minimum temperature threshold for the development of *E. lanigerum* was estimated at 4.2°C in both Palestine and France (Bodenheimer 1947, Bonnemaison 1965) and 5.2°C (Asante *et al.* 1991) in Australia. Bo & Rongping (1989) found a higher minimum threshold temperature of 10.54°C in China. In Australia the temperature for optimum fecundity, survival and intrinsic rate of population increase was between 13 and 25°C (Asante *et al.* 1991). Temperatures above 25°C were detrimental. This supports findings of Marcovitch (1934) who reported that 20°C was near the optimum for growth and development and 30°C was unfavourable. In Palestine the optimum temperature for *E. lanigerum* was between 16 and 20°C (Bodenheimer 1947). Walker *et al.* (1988) found that the reproductive rate peaked at 24°C and maximum fecundity occurred at 16°C. They also found that with alternating temperatures, as occurred in the orchards, *E. lanigerum* development was more rapid than at a constant temperature of 30°C. The number of progeny, reproductive period and longevity were all influenced by temperature (Baker 1915, Marcovitch 1934, Bodenheimer 1947, Evenhuis 1958, Bonnemaison 1965, Gautam & Verma 1983). The number of generations per year on apple trees is usually from 10 to 14 (Evenhuis 1958, Bonnemaison 1965, Asante 1994 a), although Marcovitch (1934) recorded 18 generations in the USA.

Damage to the aerial parts

E. lanigerum is a bark feeder. It infests the roots, tender areas on the trunk and branches, new lateral growth and where the bark has been damaged, either

accidentally, during pruning or by hail (Schoene & Underhill 1935, Gambrell & Young 1950, Georgala 1953, Carnegie 1963, Asante *et al.* 1993).

Feeding on the roots and shoots causes the formation of galls (Le Pelley 1927, Georgala 1953, Brown & Schmitt 1990, Asante *et al.* 1993). These can be invaded by fungi when the galls burst and the wood is exposed, causing apple canker, *Nectria ditissima* Tul. and *Nectria galligena* Bres. (Baker 1915, Childs 1929, McLarty 1933, Rinallo *et al.* 1995). Large galls in high numbers can cause die-back of affected twigs and branches (Georgala 1953).

Feeding above ground in pruning-cuts, wounds, or leaf axils can reduce tree vigour and interfere with wound closure (Childs 1929, Madsen & Bailey 1958 in Brown *et al.* 1991, Nel 1983). The fruit in the vicinity of infestations can become sticky with honeydew on which black sooty mould grows, resulting in the down grading of the fruit (Nel 1983). Some aphids have also been found in apple cores which can be detected in apple juice or canned fruit (Essig 1942, Madsen *et al.* 1954, Madsen & Hoyt 1957). The sooty mould on the leaves can also hinder photosynthesis and respiration (Georgala 1953).

The galls contain higher nitrogen levels than other wood, resulting in an increased nutritional status for *E. lanigerum* (Brown *et al.* 1991). This makes them beneficial to the insect, resulting in an increased susceptibility to further infestation (Miles 1972).

Control of the aerial populations

Biological control

The most important parasitoid for the biological control of woolly apple aphid is the chalcid wasp, *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) (Lundie 1924, Bodenheimer 1947, Evenhuis 1958, De Bach 1964). Lundie (1924) gave a detailed account of the development of *A. mali*. The parasitoid deposited one egg per aphid, and in the rare cases where more than one was deposited, only one matured. At first the developing larva that emerged from the egg was long and narrow. As the larva grew it became broader than long. When the aphid was killed the body turned black and was attached to the twig or other parasitised aphids by a liquid that oozed from the aphid through the body wall where it was connected to the twig. The pupa was uniformly yellow with reddish brown eyes. The adult appeared through a round hole made in the dorso-caudal region of the aphid's abdomen. *A. mali* overwintered as a full grown diapausing larva (Lundie 1924, Evenhuis 1958, Trimble *et al.* 1990) inside the hardened shell of its host. Most larvae terminated diapause by the end of winter (Trimble *et al.* 1990) and the larvae stayed in postdiapause until temperatures higher than 9.4°C (theoretical lower threshold temperature) were encountered (Trimble *et al.* 1990).

The number of eggs laid by each female, development time as well as longevity were dependent on temperature. Between 48 and 140 eggs were laid by one female which suggested great variation even between females under the same conditions (Lundie 1924). However, Lung *et al.* (1960) found that females from different places showed differences in their reproductive potential and their longevity.

A. mali is relatively free of hyperparasitoids. The hyperparasitoids *Pachyneuron aphidis* (Bouché), *Asaphes vulgaris* Walk were found in the Netherlands (Evenhuis 1958). *Ceraphron sp., P. aphidis* and *A. vulgaris* were also found to be hyperparasites in Russia (Meier & Telenga 1933). Lundie (1924) found *A. americana* and a *Pachyneuron sp.* hyperparasitising *A. mali*. However, all these hyperparasites were found in such low numbers that they were considered to be of little importance.

A. mali was introduced in Southern Africa from North America in 1920 (Lundie 1939) and the level of control of *E. lanigerum* was so high that spraying against the pest was no longer necessary. However, Lundie (1939) stated that there were factors, such as the weather that may retard the activity of the parasitoid. *A. mali* has been established in at least 40 countries where it has been reported as being a successful biological agent (DeBach 1964).

Biological control of *E. lanigerum* varies from very good to poor in different countries. Reasons for poor or partial control are usually ascribed to the weather. In warmer apple producing regions, *A. mali* has usually provided complete or at least substantial control. However, control has been insufficient in many colder areas (DeBach 1964). Temperature is one of the most important physical factors known to have a differential effect on the development of aphids and their parasitoids (Campbell *et al.* 1974). *E. lanigerum* has a higher developmental rate than *A. mali* (Bodenheimer 1947, Evenhuis 1958, Lung *et al.* 1960, Bonnemaison 1965, Walker *et al.* 1988, Asante & Danthanarayana 1992). This is not unusual for a host-parasitoid relationship (Campbell *et al.* 1974). By developing more slowly than their hosts, some parasites ensure the continued availability of a minimum host supply and thus their own survival. At low and intermediate temperatures *E. lanigerum* matured more rapidly than *A. mali* (Walker *et al.* 1988). Thus *E. lanigerum* individuals may escape

biological control during cool periods. The estimated lower temperature threshold for development of *E. lanigerum* was always lower than that of *A. mali* (Bodenheimer 1947, Bonnemaison 1965, Asante *et al.* 1991). The threshold for *A. mali* was estimated at 8.6°C in Palestine (Bodenheimer 1947), 8.2°C in France (Bonnemaison 1965), 8.3°C in Australia (Asante & Danthanarayana 1992) and approximately 12°C in the Netherlands (Evenhuis 1958). Walker *et al.* (1988) gave the lower temperature threshold as 6.4°C for the *A. mali* larvae and 9.4°C for the pupae. Trimble *et al.* (1990) also found that the lower temperature threshold for post-diapausing *A. mali* was 9.4°C. These minimum threshold temperatures are higher than those of *E. lanigerum.*

Lung *et al.* (1960) and Evenhuis (1962) found that when *A. mali* appeared during spring after the termination of diapause they could not find sufficient hosts which can reduce the *A. mali* population levels. In addition, early season sprays against other apple pests, such as codling moth, could further reduce *A. mali* numbers (Bengston 1960, Lower 1968, Hely *et al.* 1982). These factors could have a negative effect on the efficacy of parasitisation of *E. lanigerum* early in the season.

Up to 1944/45, before the era of synthetic organic insecticides, the spray programme against codling moth, *Cydia pomonella* (L.) consisted of a series of lead arsenate or fixed nicotine sprays in Southern Africa (Georgala 1953). These sprays did not affect the activity of *A. mali,* and sprays like fixed nicotine and summer oil had some toxic effect on the aphid. With the introduction of DDT codling moth was controlled but other pests, such as *E. lanigerum* became numerous (Georgala 1953). DDT had no effect on the aphid, but was highly toxic to *A. mali* (Newcomer *et al.* 1946, Newton & List 1952, Schneider 1958). Yothers (1947) also reported that where *A. mali* previously controlled *E. lanigerum*, with the use of DDT the aphid threatened

to become a destructive pest again. Although parasitoid numbers increased later in the season, by the time this happened a great deal of damage had been done during the period when the trees should have formed fruit buds, accumulated reserves and ripened the wood (Georgala, 1953).

A. mali often emerged early in the spring before *E. lanigerum* became abundant (Lung *et al.* 1960, Evenhuis 1962) and population numbers were reduced because of a shortage of hosts for oviposition. However, *A. mali* in diapause or in the post-diapause state can survive in cold storage for long periods (Lundie 1939, Trimble *et al.* 1990). As postdiapause *A. mali* respond immediately to temperatures which are suitable for growth and development (Trimble *et al.* 1990) there is a possibility that mummies of *E. lanigerum* containing post-diapause larvae can be kept in cold storage and be placed in orchards after detrimental chemical sprays have been applied and when there are sufficient *E. lanigerum* available.

The activity of the natural enemies can also be enhanced by encouraging plants in orchards which are rich in nectar, such as *Phacelia* spp. and *Eryngium* spp. (Van den Bosch & Telford 1964). The per cent parasitism by *A. mali* was also higher on trunks and branches when there was less pruning and in the presence of a suitable cover crop (El-Haidari *et al.* 1978). On the other hand, pruning may reduce *E. lanigerum* population levels by removing many of the overwintering colonies in broken galls on the one year old wood, and by keeping the tree open to wind and sun (Greenslade 1936).

There are many other natural enemies of *E. lanigerum* and a review is given by Asante (1997). However, few of them control *E. lanigerum*. Among the effective predators are *Syrphus confrator* Wiedmann and *Chrysopa scelestes* (Banks), which have replaced *A. mali* as the most important natural enemy in areas of India (Thakur

et al. 1988). In Southern Africa larvae of syrphid flies and adults and larvae of the coccinellid *Exochomus flavipes* (Thunb.) have been seen preying on *E. lanigerum* (Geyer 1947, Carnegie 1963).

Chemical Control

E. lanigerum became a pest when synthetic pesticides came into use with the result that it also had to be controlled by spraying (Smith 1964). Its control in the Western Cape Province was combined with that of scale insects. Sprays affecting *E. lanigerum* consisted of a dormant application of lime-sulphur and winter oil at about the end of August, plus parathion applied against codling moth at the end of December. In severe cases, a second spray, usually metasystox, was applied during the middle of January (Smith 1964).

In recent years vamidothion (Kilval) was recommended for the control of *E. lanigerum* as it gave excellent control (Swart *et al.* 1990). However, the aphid has recently become more of a problem in the Elgin area as a result of resistance to this chemical (Pringle *et al.* 1994). In Israel severe outbreaks of *E. lanigerum* have also occurred after the use of vamidothion, the only recommended insecticide for this pest in that country (Cohen *et al.* 1996).

E. lanigerum is not easily controlled by the usual routine sprays as they are protected by a tangled mass of wax threads. The aphids, even at very low densities, tend to aggregate (Asante *et al.* 1993) which protects some individuals against chemical sprays. *E. lanigerum* also feeds in wounds that provide protection against sprays. As these aphids reproduce parthenogenetically a single female can start a

colony and together with the high rate of reproduction makes chemical control of *E. lanigerum* difficult (Greenslade 1936).

Materials which not only controlled aphids in the aerial parts of the tree, but also prevented upward movement, were capable of providing better long term control than materials which gave high initial kill, but with little residual effect (Madsen & Hoyt 1957).

Cultural measures

Large numbers of wandering young nymphs are caught on grease bands, but unless there is a definite migration upwards from the roots, grease banding alone would not be practical (Le Pelley 1928 in Greenslade 1936). Irrigation can also prevent migration into the trees to a large extent (Greenslade 1936) in areas of heavy soil because it could prevent cracking and so reduce root infestation.

Integrated control

It is clear that there is presently no single method that can be used to control *E. lanigerum*. The different methods have to be integrated to get the best results, like the integration of chemical control with biological control. To enhance biological control chemicals for the control of diseases and other pests that are relatively non-toxic to the parasitic *A. mali* may be used, or sprays can be applied when the parasitoid is not active. In some cases *A. mali* larvae could survive chemical sprays inside mummies of the dead aphids (Hameed *et al.* 1974, El-Haidari & Georgis 1978, Rawat *et al.* 1988). Hameed *et al.* (1974) found for example that vamidothion gave effective control of *E.*

lanigerum while 74.9 % *A. mali* still emerged from treated mummies. However, Cohen *et al.* (1996) found that the long persistent effect of vamidothion on field populations of *E. lanigerum* caused the disappearance of *A. mali*. As a result a second application of vamidothion was needed. The short-term effect of triazamate resulted in the initial reduction of the *E. lanigerum* populations and preservation of the parasitoid in the field, which was then able to control the recovering population (Cohen 1994 in Cohen *et al.* 1996).

Present study

In recent years *E. lanigerum* became more important as a pest of apples in the Elgin area of the Western Cape Province. With the development of resistance of *E. lanigerum* to certain chemicals (Pringle *et al.* 1994) and the loss of others due the export restrictions, as well as the apparent inability of *A. mali* to control the aphid successfully, it became important to gain insight into the biology of *E. lanigerum* and *A. mali*. An intensive investigation of *E. lanigerum* was planned. Damavandian (2000) studied subterranean populations while the present investigation concentrates on above ground populations of *E. lanigerum* and *A. mali.* The following aspects were included in the study:

1. A method to distinguish between the different developmental stages of *E. lanigerum.*
2. The effect of constant temperatures on the development, growth and reproduction of laboratory reared *E. lanigerum*.
3. The seasonal development of populations of *E. lanigerum* and its natural enemy, *A. mali*, in apple orchards.

4. A sampling system for the effective monitoring of *E. lanigerum* to assist in the decision making regarding chemical intervention.
5. The influence of chemicals high in nitrogen on the ability of *E. lanigerum* to colonise apple trees.
6. The influence of barriers for the control of the fruit weevil, *Phlyctinus callosus* Boh., on population levels of *E. lanigerum* in apple trees.
7. The development of *A. mali* at constant temperatures as well as the development and survival of postdiapause *A. mali* after extended periods of cold storage. The optimum time for collecting postdiapause *A. mali* from the orchard and the influence of different temperatures on the development of *A. mali* when diapause was completed.
8. The age structure within colonies of *E. lanigerum* through the season and within colony parasitism by *A. mali.*
9. The susceptibility of *A. mali* to different insecticides used in commercial apple orchards.

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CHAPTER 2

THE IDENTIFICATION OF THE DEVELOPMENTAL STAGES OF *ERIOSOMA LANIGERUM* (HAUSMANN).

2.1. Introduction

Reliable criteria for age-grading field-collected woolly apple aphid, *Eriosoma lanigerum* (Hausmann), are of critical importance for development and interpretation of time-specific life table data as well as for the development of pest management systems. For example, if small, early instar aphids are attacked by the parasitic wasp *Aphelinus mali* Hald. the progeny are mainly males, while wasps developing in larger, older individuals produce a higher proportion of females (Asante & Danthanarayana 1992, 1993, Mueller *et al.* 1992). Therefore, the reproductive potential of the parasitoid population can largely be determined by knowing the age structure of the aphid population.

In the present study the morphological characteristics which could be used to distinguish the different developmental stages of *E. lanigerum* were investigated.

2.2. Materials and methods

E. lanigerum colonies were collected every four weeks on twigs with visible colonies from two sites, Oak Valley (34.9 S 19.2 E) and Molteno (34.10 S 19.3 E) in the Elgin area (see chapter 4). The twigs were transported to the laboratory where the aphids were removed and placed in glycerin. Aphids were then roughly catagorised into instars based on the proboscis length relative to the body length (Baker 1915, Gautam & Verma 1987), the shape of the abdomen and the number of antennal segments, which increases from five to six supposedly after the first moult (Asante & Cairns 1995) or after the second moult (Baker 1915, Gautam & Verma 1987). A maximum of five individuals in each developmental stage was examined from each site on each sampling date. The following structures were measured using an ocular micrometer attached to a microscope: body length, body width, distance between the antenna, length of the third antennal segment, length of the newly divided antennal segment (when present) and second last antennal segment, distance between the eyes, length of the last segment (tip) of the proboscis, distance between the cornicles, cornicle diameter, anal plate width, length of the hind tibia and femur. There is confusion on the position of the cauda. According to Gautam & Verma (1987) the most posterior segment of the abdomen is known as the cauda. However, the structure Baker (1915) illustrated as the anal plate was situated at the end of the abdomen with the cauda on the dorsal surface of the abdomen before the anal plate. The cauda measured by Asante & Cairns (1995), could therefore be the same structure as the anal plate described by Baker (1915). In the present study the anal plate described by Baker (1915) was measured. Asante & Cairns (1995) referred to this as the cauda. Measurements were made immediately after the aphids were placed in the glycerin on a glass slide. The antennal segments, proboscis tip, femur and tibia were measured

after a cover slip was placed over the body of the aphid on the slide. Body length was measured from the head to the end of the anal plate and body width was measured at the widest portion of the body.

Asante & Cairns (1995) stated that cornicles were only visible from the second instar, contradicting reports of cornicles being present in the first instar (Baker 1915, Gautam & Verma 1987). Therefore, the time at which the cornicles became visible was examined by placing 20 first instar nymphs on each of five trees of approximately 15 cm high in a growth chamber set at 25°C. Fifteen aphids were examined daily until they moulted. The presence of the cornicles, the length of the proboscis in relation to the body and the development of the antenna was noted.

In addition to these, measurements were also taken from aphids reared on small apple trees as described above. Newly born nymphs were placed on the apple trees and examined daily. The presence of exuvia indicated that a new developmental stage had been achieved. Five new born (less than one hour old) nymphs were measured, and thereafter five first instar nymphs that started feeding (approximately three days old) were measured to see if there was a difference in the size of the different structures, as described above, at this stage. The structures of five aphids in each of the subsequent instars and adult stage were also measured. The length of the proboscis in relation to the body, the presence of cornicles and presence of the newly divided third antennal segment were also noted.

The measurements of field collected aphids were analysed using a factorial analysis of variance with developmental stage, farm and date as main effects. Significant probability levels for instars would indicate differences between instars in the structure in question, while significant probability levels for date or farm would indicate that the structure in question differed between dates and farms respectively

and would, therefore, not be a suitable structure for identifying instars. In addition, interactions between the main effects would indicate that differences were not consistent between dates and farms.

Cornicles are probably only visible after the first moult and the vulva is only present in the adult female (Asante & Cairns 1995). Therefore, the lack of visible cornicles can be used to positively identify first instar nymphs and the presence of a vulva can be used to identify adults. Thus, these two developmental stages were omitted from the above analysis.

Finite mixture analysis (FMA) resolves a multimodal curve of a frequency distribution into its individual normal distribution components and calculates the proportions for each component in areas of overlap *i.e.* it calculates the normal distribution curve for each peak in a frequency distribution (Flury & Randall 1995). This model was used to identify characters producing reliable discrimination between the second and third and third and fourth instars of *E. lanigerum.* As there was no difference between the field and laboratory reared aphids all measurements were included. For the FMA analysis the FMA-N1© software written by Dr. John Randall (Department of Economic Agriculture, University of Stellenbosch, Stellenbosch, South Africa) was used. Discriminant analysis was used to quantify the precision with which individuals were classified into the correct group (instar).

2.3. Results and discussion

During the 1996/97 season *E. lanigerum* colonies were present in the orchards from February on both sites, but on Oak Valley they disappeared after April. Therefore, aphids for measurements were collected only from Molteno for the remainder of the season.

There were no visible cornicles on first instar nymphs as reported by Baker (1915) and Gautam & Verma (1987). Cornicles were observed on all laboratory reared second instar nymphs (Table 2.1), conforming to the observations of Asante & Cairns (1995) in Australia. However, the criteria originally used for classifying *E. lanigerum* into instars did not include the presence of cornicles and therefore some aphids without cornicles were classed as second instar. The length of the proboscis in relation to the abdomen of second instar aphids differed, but was usually found to extend to the tip of the abdomen or at least beyond the third coxae. Two aphids had proboscises shorter than the distance to the third coxae (Table 2.1). The development of the division of the third antennal segment was variable (Table 2.1). The third segment in most cases was not divided, except for one individual which had one antenna with six segments and one with five segments. A dividing furrow was not even visible in the antenna with five segments.

Table 2.1. Presence of cornicles, proboscis length in relation to the abdomen and the structure of the third antennal segment in laboratory reared second instar *Eriosoma lanigerum*.

Cornicles		**Proboscis length**		**Third antennal segment**	
Not visible	0	To tip of abdomen	7	Smooth	3
Visible	15	Beyond 3rd coxae	6	Slightly knobbed	6
		Up to 3rd coxae	1	Dividing furrow visible	5
		Up to 2nd coxae	1	1 knobbed, 1 divided	1

Division of the third antennal segment could not be used to distinguish between any of the developmental stages (Table 2.2) in laboratory reared aphids. The

antennae of all the first, second and third instar aphids had only five segments. In addition, only a few of the fourth instar and adults had six antennal segments. In field populations some third instar aphids had six antennal segments, but some fourth instars and even a few adults (with visible vulva) had incomplete division of the third segment. Some of the adults from field populations had one antenna with six visible segments, the other with only a partly divided third segment or one with a faint dividing furrow.

Table 2.2. Division of the third antennal segment and length of the proboscis in relation to the abdomen in laboratory reared *Eriosoma lanigerum*.

Instar	**Antenna**		**Proboscis length**	
1	Divided	0	Past abdomen	3
(n=10)	Not divided	10	Up to tip of abdomen	2
			Past 3rd coxae	5
2	Divided	0	Up to tip of abdomen	2
(n=5)	Not divided	5	Past 3rd coxae	3
3	Divided	0	Past 3rd coxae	3
(n=5)	Not divided	5	Up to 3rd coxae	1
			Past 2nd coxae	1
4	Divided	2	Past 3rd coxae	2
(n=5)	Not divided	3	Up to 3rd coxae	2
			Past 2nd coxae	1
5	Divided	1	Past 3rd coxae	1
(n=5)	Not divided	4	Up to 3rd coxae	1
			Past 2nd coxae	2
			Past 1st coxae	1

The length of the proboscis in relation to the body of the different developmental stages showed considerable variation. The proboscis of the second instar laboratory reared aphids (Table 2.2) all extended beyond the third coxae. However, some second instar aphids collected from the orchard had a proboscis which was much shorter, not even reaching the third coxae. Subsequent instars and adults had proboscises of different lengths, extending from just beyond the first coxae to well beyond the third coxae. Therefore, this character will not be reliable for distinguishing between the different instars of *E. lanigerum* in South Africa, as was found in the U.S.A. (Baker 1915) and India (Gautam & Verma 1987).

There was a visible increase in the sizes of all the structures measured as the age of the aphids increased (Table 2.3 and 2.4). These differences were highly significant (Table 2.5). However, there were also a number of differences between dates (Table 2.5), indicating that the sizes of some of the characters measured changed during the year. In addition, body width and the distance between antenna were not the same on the two farms (Table 2.5).

The date x instar interactions for body width and distance between eyes were also significant, indicating that differences between instars were not the same on all dates. Similarly, differences in the length of the third antennal segment between instars were not consistent between the two farms confirming observations described above. The only characters for which there were differences between instars, no differences between dates or farms and no interactions were the body length, the new (fourth) antennal segment, cornicle width, femur length and anal plate width. The new antennal segment was not considered a reliable character, as it did not always appear during the same instar and sometimes only appeared on one antenna (Table 2.2).

Table 2.3. Mean (±SE) sizes (in mm) of morphological structures of the stages of field collected apterous virginoparae of *Eriosoma lanigerum*.

Structure	Instar 1	Instar 2	Instar 3	Instar 4	Adult
Body length	0.642(±0.088)	0.782(±0.078)	0.976(±0.099)	1.367(±0.187)	1.725(±0.218)
Body width	0.287(±0.039)	0.350(±0.048)	0.552(±0.097)	0.761(±0.129)	1.039(±0.191)
3rd antennal segment	0.065(±0.010)	0.07(±0.013)	0.073(±0.018)	0.085(±0.023)	0.112(±0.027)
New antennal segment			0.034(±0.012)	0.040(±0.007)	0.042(±0.01)
Sec last antennal segment	0.051(±0.007)	0.053(±0.006)	0.057(±0.006)	0.061(±0.009)	0.068(±0.008)
Between eyes	0.160(±0.018)	0.176(±0.026)	0.215(±0.029)	0.260(±0.032)	0.311(±0.028)
Between antenna	0.115(±0.036)	0.117(±0.016)	0.146(±0.021)	0.165(±0.022)	0.196(±0.027)
Anal plate	0.06(±0.009)	0.069(±0.018)	0.090(±0.015)	0.114(±0.027)	0.145(±0.041)
Between cornicles		0.207(±0.035)	0.297(±0.035)	0.426(±0.061)	0.542(±0.09)
Cornicle width		0.028(±0.004)	0.036(±0.007)	0.047(±0.008)	0.058(±0.009)
Proboscis	0.121(±0.011)	0.122(±0.009)	0.130(±0.009)	0.138(±0.011)	0.158(±0.020)
Femur	0.160(±0.013)	0.172(±0.016)	0.205(±0.025)	0.248(±0.038)	0.322(±0.040)
Tibia	0.165(±0.019)	0.172(±0.018)	0.201(±0.033)	0.244(±0.047)	0.335(±0.060)

Table 2.4. Mean (±SD) sizes (in mm) of morphological structures of the stages of apterous virginoparae of *Eriosoma lanigerum* reared in the laboratory.

Structure	Instar 1	Instar 2	Instar 3	Instar 4	Adult
Body length	0.583(±0.052)	0.787(±0.127)	0.945(±0.127)	1.167(±0.197)	1.441(±0.112)
Body width	0.256(±0.025)	0.406(±0.090)	0.509(±0.084)	0.682(±0.130)	0.989(±0.088)
3rd antennal segment	0.050(±0.004)	0.077(±0.009)	0.087(±0.012)	0.081(±0.031)	0.143(±0.033)
New antennal segment				0.045(±0.017)	0.044
Sec last antennal segment	0.054(±0.006)	0.050(±0.008)	0.055(±0.004)	0.053(±0.006)	0.063(±0.004)
Between eyes	0.155(±0.009)	0.186(±0.019)	0.200(±0.015)	0.220(±0.006)	0.266(±0.010)
Between antenna	0.108(±0.015)	0.121(±0.005)	0.134(±0.009)	0.144(±0.021)	0.184(±0.018)
Anal plate	0.052(±0.008)	0.078(±0.010)	0.093(±0.011)	0.106(±0.017)	0.180(±0.047)
Between cornicles		0.220(±0.061)	0.291(±0.052)	0.383(±0.076)	0.557(±0.028)
Cornicle width		0.029(±0.005)	0.038(±0.005)	0.048(±0.012)	0.058(±0.005)
Proboscis	0.123(±0.012)	0.126(±0.009)	0.123(±0.007)	0.126(±0.010)	0.140(±0.002)
Femur	0.152(±0.014)	0.190(±0.015)	0.201(±0.015)	0.233(±0.038)	0.326(±0.030)
Tibia	0.158(±0.016)	0.192(±0.009)	0.196(±0.012)	0.223(±0.035)	0.259(±0.148)

Table 2.5. Probability levels for differences in sizes of different structures of field collected *Eriosoma lanigerum* between date, instar and farm and for interactions between these main effects.

Structure Measured	Date	Instar	Farm	Date x instar	Date x Farm	Instar x Farm	Date x Instar x Farm
Body length	0.071	<0.001	0.256	0.343	0.318	0.321	0.247
Body width	0.080	<0.001	0.023	0.015	0.013	0.585	0.799
3rd ant. segment	0.242	<0.001	0.054	0.247	0.972	0.020	0.942
New ant. Segment	0.502	0.015	0.083	0.071	0.707	0.213	0.077
Sec last ant. Segment	0.002	<0.001	0.952	0.058	0.385	0.260	0.772
Between eyes	0.002	<0.001	0.164	0.012	0.114	0.068	0.276
Between antenna	<0.001	<0.001	0.002	0.501	0.074	0.508	0.770
Anal plate	0.261	<0.001	0.783	0.732	0.526	0.396	0.575
Between cornicles	<0.001	<0.001	0.120	0.432	0.023	0.278	0.726
Cornicle width	0.080	<0.001	0.662	0.731	0.146	0.654	0.607
Proboscis	<0.001	<0.001	0.332	0.157	0.115	0.631	0.194
Femur	0.220	<0.001	0.890	0.685	0.413	0.063	0.770
Tibia	<0.001	<0.001	0.842	0.316	0.061	0.680	0.523

Therefore, the only reliable characters that may be used for identifying instars 2, 3 and 4 were body length, cornicle width, femur length and anal plate width. This partly supported Asante & Cairns (1995) who found that the distance between cornicles, cauda width, body length and hind tibia length were the most reliable criteria for discrimination between instars.

Body length, cornicle width, femur length and anal plate width were analysed using finite mixture analysis. Three categories (instar 2, 3 and 4) were specified. Body width was also included in the finite mixture analysis, despite the interactions between date and instar as well as date and farm, because Damavandian (2000) found it to be suitable for distinguishing between instars in subterranean *E. lanigerum.* Asante & Cairns (1995) found the distance between the cornicles as one of the most important characteristics to distinguish between the instars. Therefore this character was also included in the finite mixture analysis. The presence of the vulva in the adult *E. lanigerum* was not initially used to distinguish between the fourth instar and adult stage. Therefore, some aphids with vulvas were classed as fourth instars. Some aphids initially classified as first instar nymphs could also have been included, as the presence of the cornicles was not initially used to distinguish between second and first instar nymphs as described earlier.

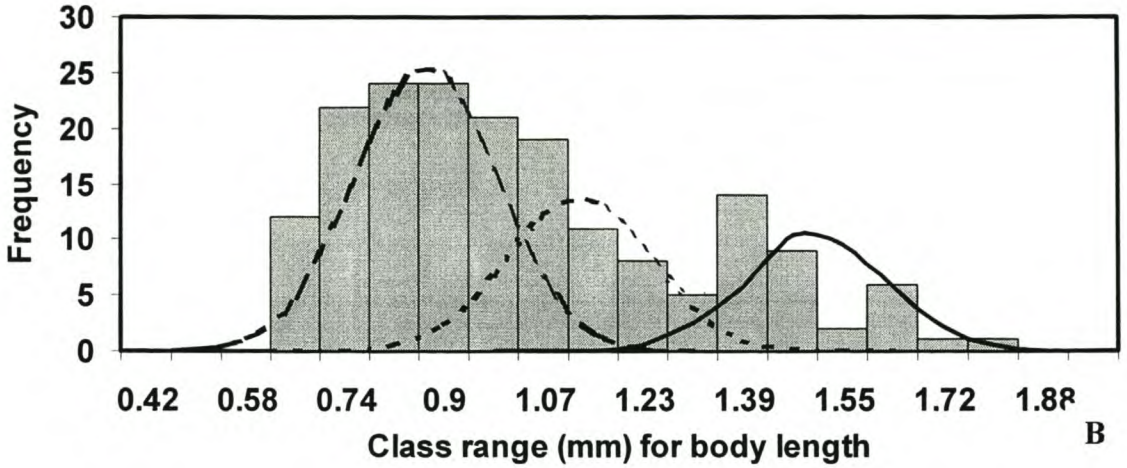
Convergence was achieved for all these characters (Table 2.6) in the finite mixture analysis. In the case of femur length and anal plate width very low proportions of individuals were classified as 4^{th} instar (Table 2.6), casting doubt on the usefulness of these characters for classifying *E. lanigerum* into instars. The proportions obtained from the finite mixture analysis for cornicle width also seemed incorrect as the largest proportion was classified as second instars (Table 2.6). However, originally cornicle width was measured on only a few second instar aphids as in the original classification some first instar aphids were classified as second instar for reasons given in Material and Methods. The frequency distributions of measurements recorded for each structure and the estimated frequency distributions of the three groups (instar 2, 3 and 4) obtained from the finite mixture analysis are illustrated in Fig. 2.1. The estimated frequency distribution for body length (Fig.

2.1A) and body width (Fig. 2.1B) divided clearly into three groups (instars). However, there were large areas of overlap between instars 2 and 3 and between instars 3 and 4. There was a more even distribution of individuals between the three groups (instars) using measurements of body width than of body length.

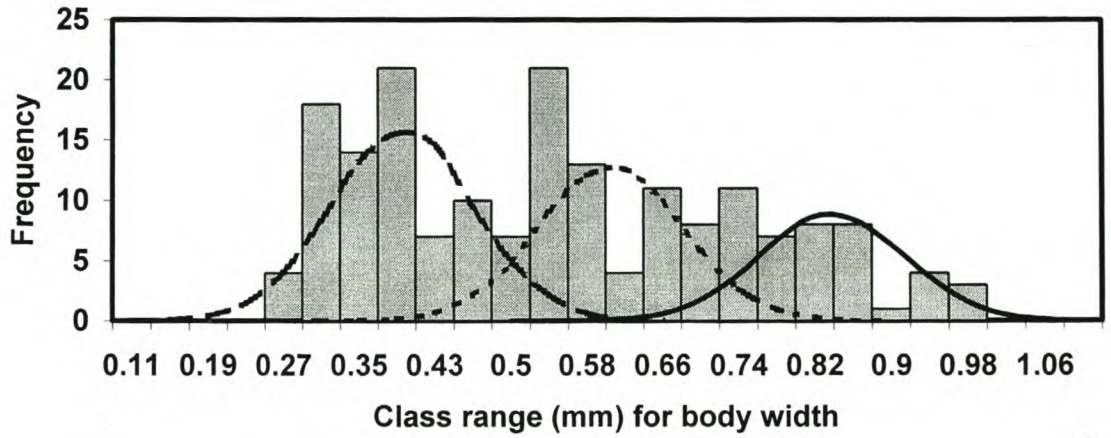
Table 2.6. Proportion of *Eriosoma lanigerum* classified into instar 2, 3 and 4 with the average measurements and variances obtained from the finite mixture analysis.

Structure	Instar	Proportion	Average measurement	Variance
Body	2	0.5150	0.8333	0.031053
length	3	0.2785	1.0827	
(n=179)	4	0.2065	1.4672	
Body width	2	0.4205	0.3728	0.005512
(n=179)	3	0.3422	0.5875	
	4	0.2374	0.8162	
Between	2	0.1841	0.2194	0.002077
cornicles	3	0.4857	0.3052	
(n=139)	4	0.3303	0.4475	
Femur	2	0.6888	0.1879	0.000509
length	3	0.2733	0.2409	
(n=165)	4	0.0379	0.3304	
Cornicle	2	0.4795	0.0321	0.000023
width	3	0.4085	0.0438	
(n=-114)	4	0.1119	0.0575	
Anal plate	2	0.5742	0.0734	0.000174
width	3	0.3532	0.1071	
(n=178)	4	0.0726	0.1490	

A



B



C

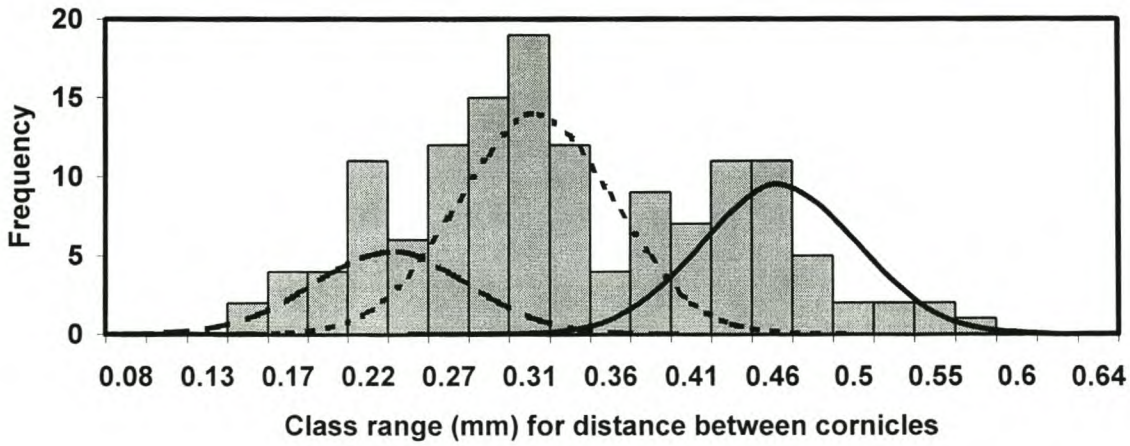


Fig. 2.1. Frequency distribution for body length (A), body width (B) and distance between cornicles (C) of *Eriosoma lanigerum* (bars), and the estimated frequency distribution of instars 2, 3 and 4 obtained from the finite mixture analysis. Broken line = 2nd instar; dotted line = 3rd instar and solid line = 4th instar.

Although the estimated frequency distribution of the distance between cornicles (Fig. 2.1C) divided into three groups (instars), the second instar had a lower estimated frequency and there was a larger overlap between the second and third instar than between the third and fourth instar. The smaller number of second instar aphids of which the distance between the cornicles was measured could explain this. Therefore, this division of frequency distributions could be correct.

The initial stepwise discriminant analysis (Table 2.7) included the structures measured in the following order; between cornicles, body length, third antennal segment, hind femur, cornicle width, body width. Using these characters there was 90.63 % agreement with the original classification into instar 2, 3 and 4.

However, in the finite mixture analysis using hind femur and cornicle width the proportions obtained seemed incorrect. Therefore these characters were omitted from the discriminant analysis. No convergence was achieved in the finite mixture analysis using the third antennal segment. Therefore, this character was also omitted. Body width was also omitted, as there were interactions between date and instar as well as date and farm. In the new discriminant analysis using only the distance between cornicles and body length (Table 2.8) there was 89.38% agreement with the original classification into instars 2, 3 and 4.

In the first discriminant function a relatively large standarised coefficient as well as a high correlation with both measurements were obtained (Table 2.9). Although the standardised coefficients of the second discriminant function were also relatively high a much lower correlation was observed (Table 2.9). The first discriminant function represented 98.73% of the variance. Therefore, most of the separation with these two measurements was obtained with the first discriminant

function. This is supported by the plot of the second discriminant function against the first discriminant function (Fig. 2.2). This graph showed that more separation between instars 2, 3 and 4 occurred in the horisontal plane (first discriminant function) than in the vertical plane(second discriminant function). Figure 2.2 also shows that there was some overlap between instars 2 and 3 and between instars 3 and 4. The classification functions that can be used for classifying aphids into instars are given in Table 2.10.

Table 2.7. Initial stepwise discriminant analysis of measurements of *Eriosoma lanigerum* for classification of *E. lanigerum* into instars.

	Step	F to entr/rem	df 1	df 2	p-level
Between cornicles	1	124.9655	2	108	<0.001
Body length	2	8.7376	2	107	<0.001
3rd Antennal segment	3	3.3539	2	106	.038695
Femur	4	3.2314	2	105	.043461
Cornicle width	5	2.3088	2	104	0.104456
Body width	6	1.7152	2	103	0.185022

Table 2.8. Stepwise discriminant analysis of selected measurements of *Eriosoma lanigerum* for the classification of instars.

	Step	F to Entr/rem	df 1	df 2	p-level
Between cornicles	1	153.1108	2	136	<0.001
Body length	2	12.4582	2	135	<0.001

Table 2.9. Standardised coefficients and correlation of the characters with the two discriminant functions.

Characters	Discriminant function 1		Discriminant function 2	
	Standardised Coefficients	Correlation	Standardised Coefficients	Correlation
Between cornicles	-0.553	-0.908	-1.191	-0.418
Body length	-0.549	-0.907	1.193	0.421

Table 2.10. Classification functions for identifying between instars 2, 3 and 4 of *Eriosoma lanigerum* using measurements of the distance between cornicles and body length.

Character	Instar 2	Instar 3	Instar 4
Between cornicles	11.7208	39.2005	61.1732
Body length	34.5893	36.1746	48.4394
Constant	-16.9325	-24.3353	-46.4833

The posterior probability that an aphid will be classified into one of the three groups (instars) from a measurement of body length is given in Fig. 2.3A and a measurement of the distance between cornicles in Fig. 2.3B. Both of these show overlap between the instars, which supports the frequency distribution given by the finite mixture analysis. The degree of overlap when using these characters was not as high as that obtained by Damavandian (2000). Therefore, separation into instars of the above ground population will be more precise than with the subterranean population.

As a result of these studies, it is clear that first instar *E. lanigerum* can be identified by the absence of cornicles, second, third and fourth instar aphids can be separated using the classification functions which include measurements of body length and distance between cornicles (Table 2.10), while adult *E. lanigerum* can be identified by the presence of the vulva. Measurements of various characters of *E. lanigerum* by Asante & Cairns (1995) indicated that the Australian strain was larger than the Elgin strain. Therefore, *E. lanigerum* from different geographical areas may show differences in the size of characters.

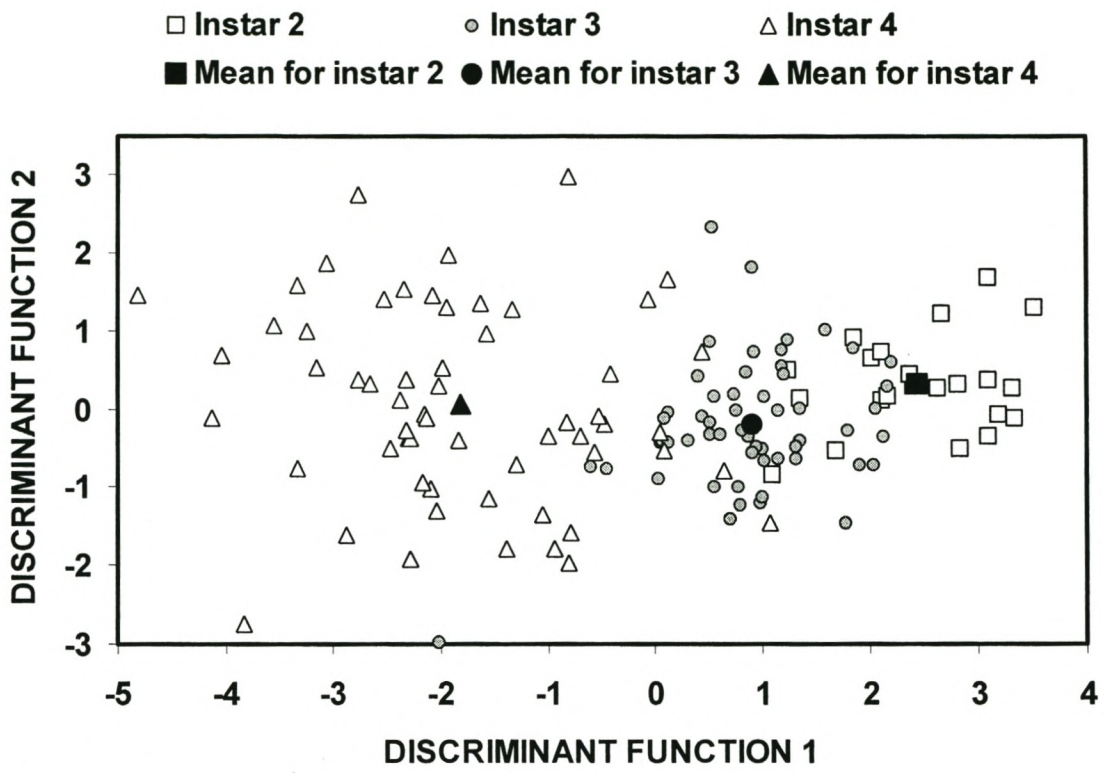


Fig. 2.2. Discriminant function 2 plotted against discriminant function 1 for 2nd, 3rd and 4th instar field collected nymphs and laboratory reared *Eriosoma lanigerum*.

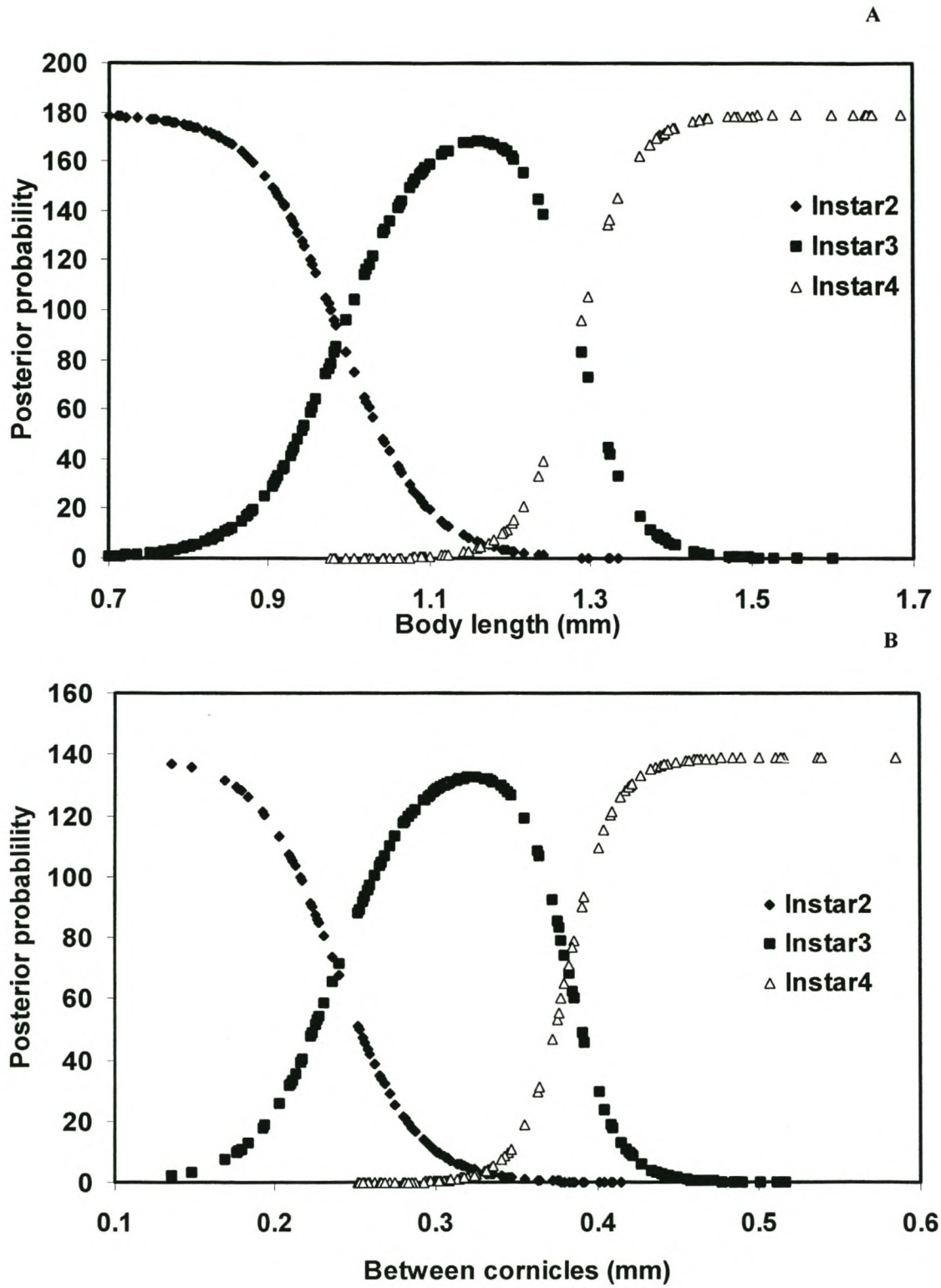


Fig. 2.3. Posterior probability curves relating body length (A) and the distance between cornicles (B) to the probability of classifying *Eriosoma lanigerum* as instar 2, 3 or 4.

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CHAPTER 3

THE INFLUENCE OF CONSTANT TEMPERATURES ON THE DEVELOPMENT OF WOOLLY APPLE APHID, *ERIOSOMA LANIGERUM* (HAUSMANN).

3.1 Introduction

The capacity of a species to increase depends on numerous environmental factors which cause fluctuations of animal populations in nature. Agricultural entomologists are particularly interested in conditions which permit insects to increase to destructive numbers (Watson 1964). Temperature is one of the most important physical factors in this regard and it is known to have a differential effect on the development of aphids and their parasitoids (Campbell *et al.* 1974).

There are conflicting reports on the developmental threshold of *Eriosoma lanigerum* (Hausmann) and the temperature at which it produced most nymphs (Asante *et al.* 1991, Bodenheimer 1947, Bonnemaison 1965, Evenhuis 1958, Walker *et al.* 1988). This may be due to experimental error or the existence of biotypes of *E. lanigerum* with different characteristics.

This study was therefore carried out to investigate developmental responses of *E. lanigerum* from the Western Cape Province of South Africa to various constant temperatures and data were gathered for the construction of a life table. Such data are

frequently used to formulate the basic survival and fecundity functions of simulation models which can be used to illustrate effects of various modifying factors on population dynamics (Gutierrez *et al.* 1972, Huffaker 1980).

3.2 Material and methods

Branches infested with *E. lanigerum* colonies were collected from commercial orchards in the Elgin district. For the first experiment at 23°C adults were removed from the branches and placed in a petri dish for ±18 hours. The first instar nymphs produced were placed on small potted apple trees (Granny Smith seedling). For the other four temperatures, 15°, 20°, 27° and 30°C newly born aphids were removed from infested branches as soon as they were born and placed on the small potted apple trees. The number of crawlers that started feeding and their location were noted. Twice a day (8h00 and 16h00) the trees were examined. The instar of each individual was noted as well as the number of crawlers each mature female had produced until she died. If exuvia or crawlers were present they were removed. Between four and six trees infested with 10 to 20 crawlers were used at each temperature. The trees were kept in incubators at the appropriate temperature until all the aphids were dead. Only aphids that died a natural death were included in the life table.

3.3 Data analysis

Developmental time, fecundity and survival data were used to construct life tables.

The net replacement rate (R_o) and the mean generation time (T) were estimated as described by Price (1984). These two values were used to obtain the initial estimate of the intrinsic rate of increase (r_m) using

$r_m = \{\ln (R_o)\}/T$ (Price 1984).

The estimates were used in the first iteration to solve the equation (Watson 1964),

$$\sum_{x-1}^{t} e^{r_m x} l_x m_x = 1 \qquad x = 1, 2, 3, \ldots t \text{ days, where}$$

x = age interval of each female,

l_x = proportion of females alive at age x and

m_x = mean number of offspring produced during age interval x.

The iterations were continued until the left-hand side of the equation was within 0.0001 of the right hand side of the equation. As *E. lanigerum* is parthenogenic with all the progeny females it was not necessary to account for the sex ratio in the life table calculations.

Developmental times of *E. lanigerum* were analysed using a factorial analysis of variance with temperature and developmental stage as main effects. The reciprocal of time to complete development (in days) was regressed on temperature. The minimum temperature for development was then estimated by solving the regression for 1/Time=0. The number of degree days required for development by *E. lanigerum* was calculated using °D=1/b, where b is the slope of the regression of 1/Time on temperature (Campbell *et al.* 1974). The upper threshold for development of *E.*

lanigerum was estimated by fitting a quadratic function of 1/Time on temperature. The first derivative was set to zero and solved for x (temperature) to give the turning point of the quadratic function. This was used as an estimate of the upper threshold temperature.

3.4 Results

3.4.1 Development

At all the temperatures nymphal development was usually completed after four instars (Baker 1915), although some moulted once more before or after they started to reproduce.

The developmental time of the first instar was longer than that of any of the following instars at all temperatures (Table 3.1) ($F_{4,536}$=409.46; P<0.001). At 15, 20, 23 and 27°C the duration of the second, third and fourth instars was very similar. The developmental times of each instar at 20, 23 and 27°C were similar. However, at 15°C the developmental times were longer than at the other temperatures, and at 30°C the developmental time of the first instar was slightly shorter than at 20, 23 and 27 °C.

3.4.2 Survival

The survival of *E. lanigerum* varied with temperature (Table 3.2.). The lowest (16.22%) and highest (80.95%) survival from birth to adult occurred at 30°C and 20°C respectively. Adult *E. lanigerum* lived the longest at 15°C and the greatest total longevity also occurred at this temperature (Table 3.2.). Longevity decreased with increasing temperature and was very low at 30°C (Table 3.2.). Constant temperatures of 30°C were unfavourable as most nymphs died before completing their

development. Some nymphs reared at 30°C never moulted and died within 20 days. This was also the longest time any aphid lived at this temperature.

Table 3.1. Developmental time of *Eriosoma lanigerum* reared at different constant temperatures. X = Average developmental time in days; SD = standard deviation; n = number of individuals.

Temp (°C)	Variable	Nymphal stadia (days)				Birth to adult
		I	II	III	IV	
15	X	8.09	4.54	4.57	4.55	21.42
	SD	1.76	1.13	0.87	0.69	2.17
	n.	27	25	23	19	19
20	X	6.44	2.60	2.47	2.66	13.94
	SD	1.42	0.57	0.51	0.38	1.54
	n.	39	36	34	34	34
23	X	6.94	2.23	2.29	2.10	13.62
	SD	1.67	0.64	0.85	0.34	1.56
	n.	25	24	21	21	21
27	X	6.17	2.44	2.18	2.36	13.2
	SD	1.40	0.64	0.32	0.33	1.74
	n.	46	41	37	35	35
30	X	5.15	2.87	3.39	3.1	12.2
	SD	2.02	1.37	1.10	0.74	2.23
	n.	27	19	13	10	10

Table 3.2. Effect of temperature on the survival of *Eriosoma lanigerum.*

Temperature (°C)	% Survival to Adult	Longevity (days±SD)	
		Adult	Total life
15	64.29	35.11±19.51	56.26±19.36
20	80.95	22.53± 9.18	35.74± 8.98
23	62.5	16.80± 7.16	29.67± 7.19
27	62.5	15.89± 6.65	28.26± 6.30
30	16.22	5.50± 2.22	17.00± 2.11

3.4.3. Fecundity

The mean number of offspring was the same at temperatures between 15°C and 23°C (Table 3.3). At higher temperatures (above 23°C) the number of offspring per female declined (Table 3.3.). The female that produced the most offspring was reared at 20°C but large numbers of nymphs were also produced at 15°C and 23°C. The number of offspring per female at each temperature was variable, especially between 15° and 23°C. At 30°C production of nymphs was low (Table 3.3.) as very few individuals reached maturity (Table 3.2). Four of the ten adults that did complete their development were unable to reproduce and the others produced between 1 and 7 nymphs each. Reproduction usually started within 24 hours after the adult appeared (Table 3.3.), except at 30°C where it took longer. At 15°C the reproductive life of females lasted longer than at any other temperature, and was shorter at 30°C (Table 3.3.) than at the other temperatures. In most cases females did not live long after reproduction ended.

Table 3.3. Fecundity and adult reproductive life of *Eriosoma lanigerum* reared at constant temperature.

Temp (°C)	n	Number of nymphs/female		Pre-reproductive life (days) ±SD	Reproductive life(days) ±SD	Post-reproductive life (days) ±SD
		Mean±SD	Range			
15	19	79.21(±39.2)	3-134	0.55(±1.09)	32.63(±16.00)	3.75(±5.84)
20	34	81.32(±36.94)	18-158	0.39(±0.72)	19.09(±5.98)	3.29(±4.46)
23	15	81.47(±34.80)	3-128	0.18(±0.53)	15.93(±6.32)	1.05(±1.25)
27	35	23.77(±11.38)	3-46	0.33(±0.47)	12.29(±4.87)	3.44(±2.85)
30	6	3.33 (±2.58)	1-7	2.5(±2.89)	1.67(±1.03)	1.17(±0.98)

3.4.4. Life table analysis

Survival rate (l_x) declined as temperature increased (Fig. 3.1 and 3.2). At 30°C the survival rate dropped drastically after 10 days (Fig. 3.2) which could be attributed to the poor survival of the first instar at this high temperature (Table 3.2). The number of female progeny per female (m_x) was more evenly spread over time at 15°C than at any of the other temperatures (Fig. 3.1). At the intermediate temperatures (20, 23 and 27°C), m_x increased from about the 10^{th} day, reaching a maximum after 15 to 20 days. This lasted for 10 to 14 days after which m_x declined drastically (Fig. 3.1). The highest number of progeny per female was recorded at 23°C. However, at the two lowest temperatures m_x was higher over a longer period of time. The result was that approximately the same average number of nymphs were produced per female at 15°, 20° and 23°C (Table 3.3). The progeny per female was low at 27°C and extremely low at 30°C indicating that fecundity is negatively influenced by these higher temperatures.

The net replacement rate (R_o) peaked at 20°C (Table 3.4) and was also high at 15°C. The intrinsic rate of increase (r_m) was highest at 20°C, with a similar value recorded at 23°C. The population doubling (D) time was slightly lower at 20 and 23°C than at 15 and 27°C (Table 3.4). It was obvious that a constant temperature of 30°C was very detrimental to *E. lanigerum,* as the doubling time was considerably longer (17.29 days) than at any of the other temperatures (Table 3.4). This could be attributed to the high mortality of first instar larvae. As was expected the mean generation time (T) decreased as the temperatures increased (Table 3.4). The time from birth until the onset of reproduction was between 14 and 15 days at all temperatures above 15°C (Table 3.4).

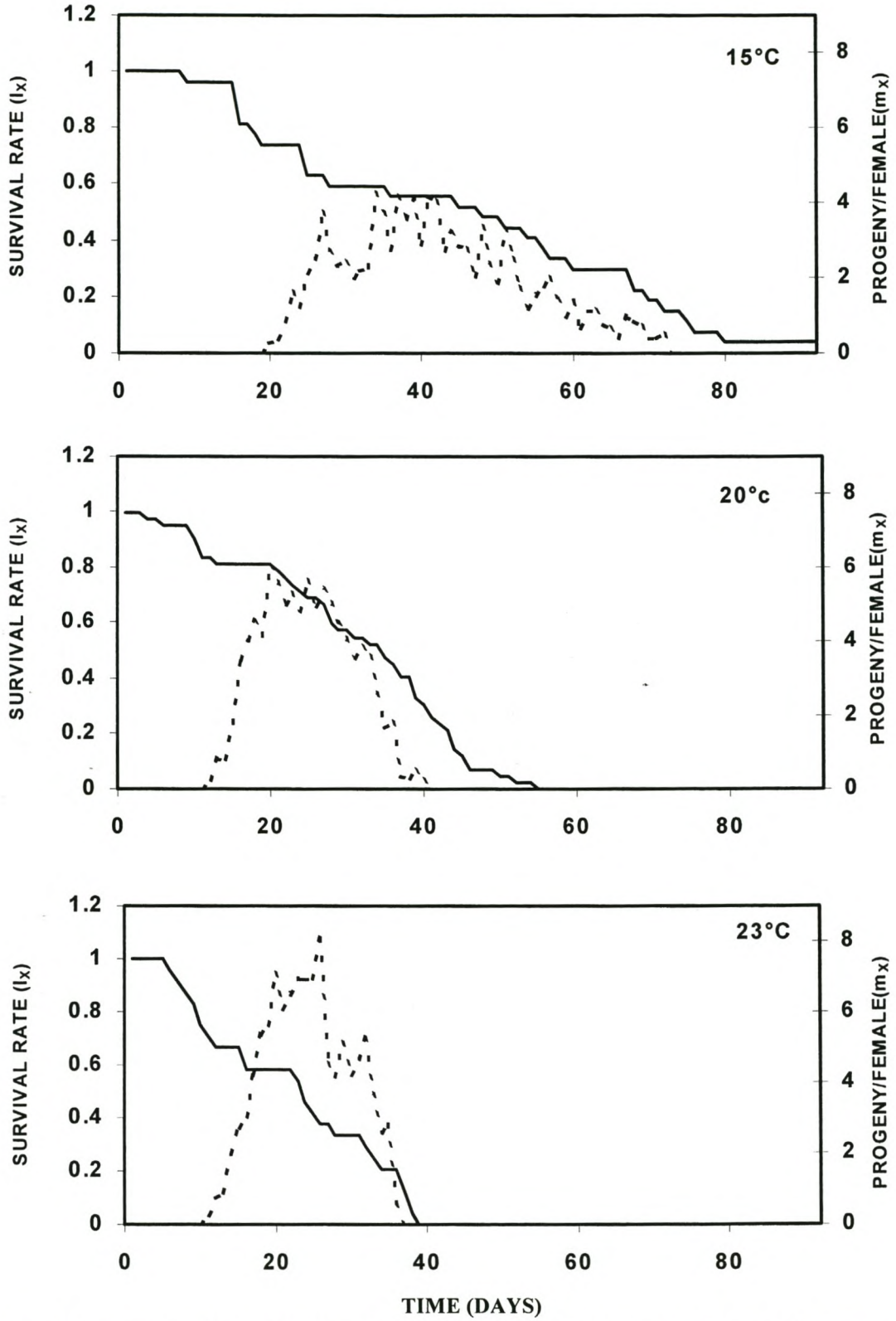

Fig. 3.1. Survival (l_x) (solid line) and fecundity (m_x) (broken line) of *Eriosoma lanigerum* at constant temperatures of 15, 20 and 23°C.

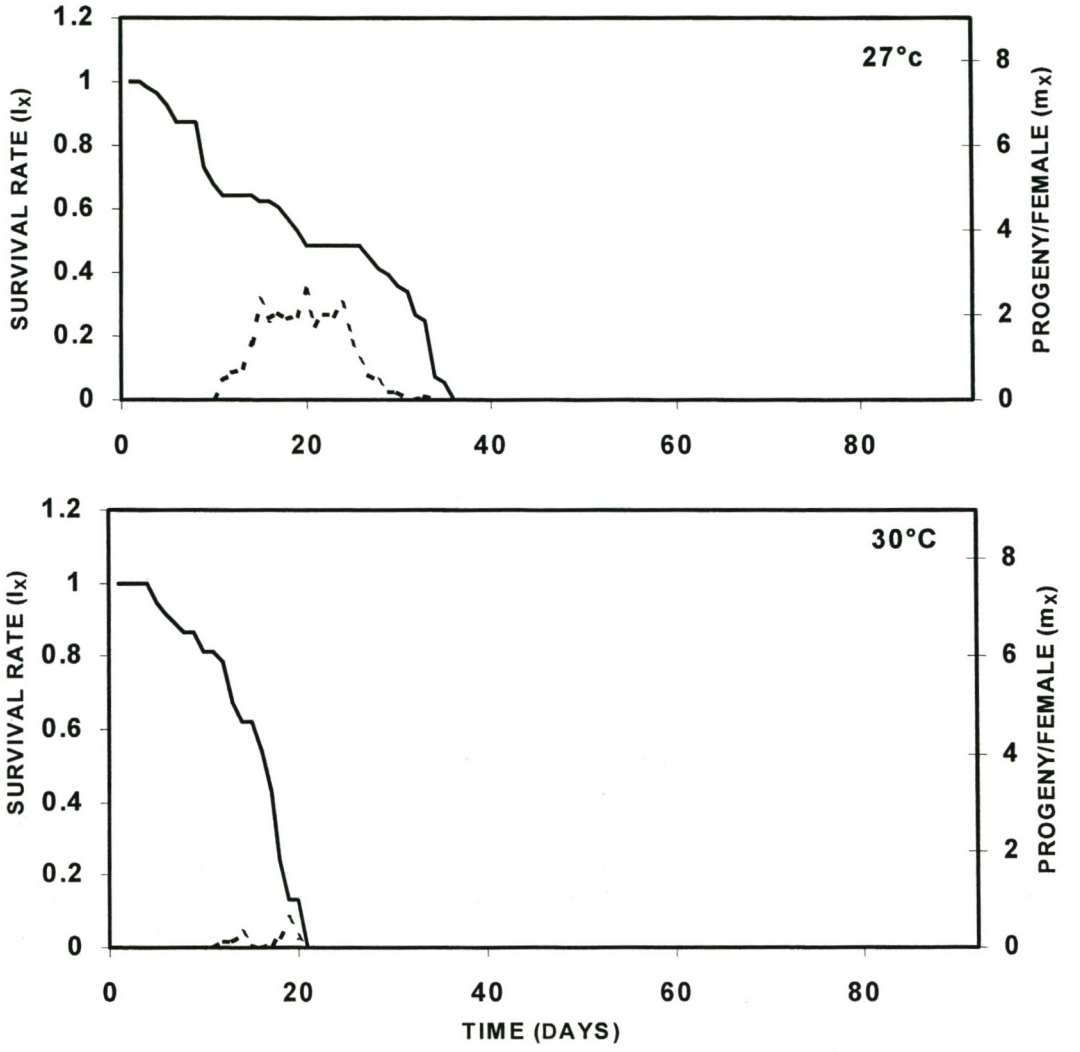

Fig. 3.2. Survival (l_x) (solid line) and fecundity (m_x) (broken line) of *Eriosoma lanigerum* at constant temperatures of 27 and 30°C.

All the data were included for computing the linear regression of 1/time on temperature to determine the lower developmental threshold (Fig. 3.3). There was a good correlation for temperatures from 15°C to 27°C (R^2=0.96). The theoretical lower temperature threshold for development was estimated at 4.48°C for total development (Fig. 3.3).

All the data were included for estimating the upper threshold limit for development. The estimated value for the upper threshold temperature was 28.07°C (Fig. 3.4). The number of degree-days (°D) required for development was calculated at 400 degree-days.

3.5. Discussion

E. lanigerum developed over a wide range of temperatures, confirming the findings of Asante *et al.* (1991). Although nymphal development was normally completed within four instars a few individuals moulted once more before or after reproduction started. A few individuals reared at the higher temperatures started reproducing after the third instar. This was also reported from Australia (Asante *et al.* 1991). Evidently the juvenile hormone level in aphids encountering unfavourable conditions, such as high temperatures, declines, resulting in the adult stage appearing after fewer instars (Blackman 1974 in Asante *et al.* 1991).

A relatively long developmental time for the first instar was also recorded in Australia (Asante *et al.* 1991), the Netherlands (Evenhuis 1958) and in Washington in the U.S.A. (Walker *et al.* 1988). However, in India almost equal duration of all the instars was reported (Gautam & Verma 1983 in Asante *et al.* 1991). Asante &

Table 3.4. Population growth statistics for *Eriosoma lanigerum* reared at five constant temperatures.

Temp (°C)	Net replacement rate (R_0)	Intrinsic rate of increase (r_m)	Doubling time (D) (days)	Mean generation time (T)(days)	Birth to beginning of reproduction (days)
15	57.89	0.1172	5.91	39.99	22.76
20	62.36	0.1965	3.53	23.64	14.78
23	50.69	0.1937	3.58	22.67	14.17
27	14.86	0.1508	4.60	19.16	14.04
30	0.54	0.0401	17.29	15.2	14.92

Danthanarayana (1990) initially thought that the relative long time spent in the first instar was due to the wandering of the newly born nymphs prior to settlement. Aphids may begin feeding on the day of birth but some are capable of searching for two days or more before they settle down to feed (Schoene & Underhill 1935, Hoyt & Madsen 1960). However, Asante *et al.* (1991) indicated that the slow development of the first instar might be a species characteristic.

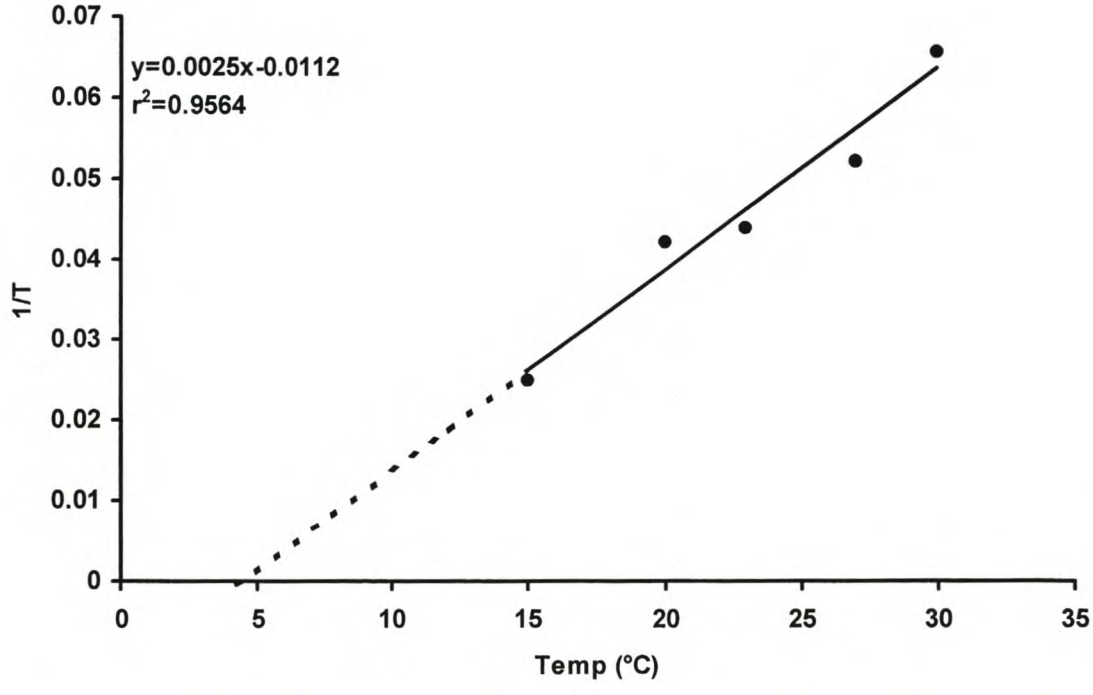

Fig. 3.3. Linear relationship between the reciprocal of mean generation time (1/T) and constant temperatures for *Eriosoma lanigerum* on aerial parts of the tree.

Fecundity of *E. lanigerum* was highest between 20°C and 23°C and lowest at 30°C, which supports the findings of Marcovitch (1934) in Tennessee, U.S.A. In China the fecundity was highest at 25°C and also lowest at 30°C (Bo & Rongping 1989). However, in Washington, U.S.A., (Walker *et al.* 1988) fecundity was highest at 16°C and in Australia it was highest between 13°C and 20°C (Asante *et al.* 1991).

Therefore, it appears as if South African populations are more productive at temperatures between 20 and 23°C than aphids in Washington and Australia. Most females began reproducing the day the aphid moulted for the fourth time, as was also found in Australia (Asante *et al.* 1991) and the Ozarks (Becker 1918).

The net replacement rate (R_o) and intrinsic rate of increase (r_m) peaked at 20°C for the South African strain. In Australia the R_o was higher than that of the South African strain at all temperatures tested and it peaked at 15°C (Asante *et al.* 1991). The r_m of the Australian strain peaked at 25°C at a lower value (0.1828) than the values obtained during this study at 20 and 23°C (0.1965 and 0.1937). The mean generation time of the local strain decreased with increasing temperature, but it was always lower than that of the Australian strain (Asante *et al.* 1991) at the corresponding temperature, indicating that the local strain developed more rapidly than the Australian one. The population doubling time was similar to that recorded by Asante *et al.* (1991) for Australia except at temperatures of 27°C and higher. At 30°C the doubling time in Australia was only 4.48 days, while it was 17.29 days in South Africa, indicating that the Australian strain tolerates temperatures better. Many of the aphids in our study did not reach maturity at 30°C, indicating that the higher temperatures were less favourable for our strain. The results obtained for the South African strain were similar to those from China (Bo & Rongping 1989) where development duration was longer at 30°C than in Australia. In Tennessee Marcovitch (1934) also found it difficult to rear the aphids at 30°C and large numbers perished. However, Walker *et al.* (1988) found that when *E. lanigerum* was reared at 30°C alternated with 20°C the survival and development of the aphids were improved.

The lower developmental temperature of 4.48°C compares well with the 4.2°C found in both Palestine (Bodenheimer 1947) and France (Bonnemaison 1965) as well

as the 5.2°C found in Australia (Asante *et al.* 1991). However, in China the lower developmental temperature was much higher at 10.54°C. The number of degree days required for development from birth to adult was much higher (400 °D) in our study than the 250 °D in Palestine (Bodenheimer 1947), 264 °D in France (Bonnemaison 1965) or the 265.96 °D in Australia (Asante *et al.* 1991). In China a much lower value of 186.98 °D was recorded. This may be explained by the low population doubling time at temperatures of 27°C and above. Campbell *et al.* (1974) explained that geographically separated populations of aphids might differ with respect to the influence of temperature on development.

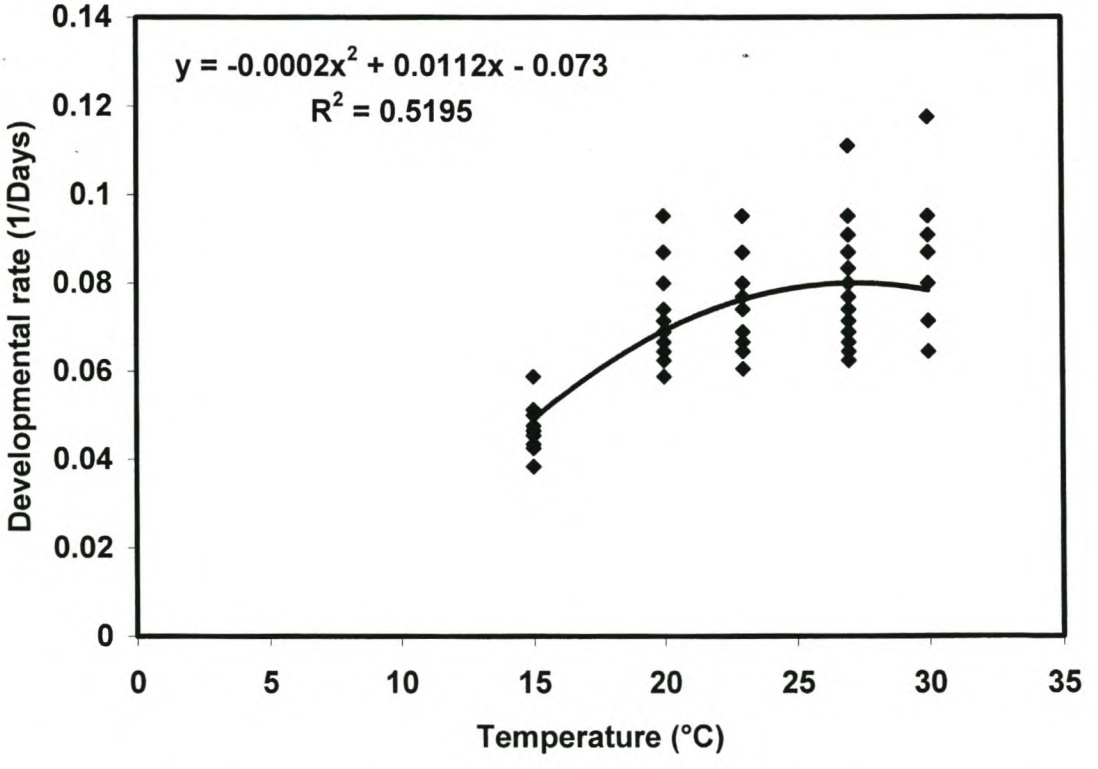

Fig. 3.4. Quadratic regression of developmental rates (1/Days) on temperature for aerial *Eriosoma lanigerum*.

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CHAPTER 4

THE BIOLOGY OF WOOLLY APPLE APHID, *ERIOSOMA LANIGERUM* (HAUSMANN), AND ITS NATURAL ENEMY, *APHELINUS MALI* (HALD.) IN APPLE ORCHARDS.

4.1 Introduction

Woolly apple aphid, *Eriosoma lanigerum* (Hausmann), is a serious pest of apple, *Malus domestica* (Borkh.), where it occurs as a bark feeder. It infests the roots, tender areas on the trunk and branches where the bark has been damaged, either accidentally or while pruning lateral shoots, as well as the seedling stem just above the variety bud, and new lateral growth (Gambrell & Young 1950, Asante *et al.* 1993). *E. lanigerum* confines its attacks to the one and two year old growth and around wounds caused by pruning, hail, etc. (Georgala 1953).

In the warmer areas, apterous virginoparae occur on apple trees throughout the year (Hoyt & Madsen 1960, Asante 1994). In New South Wales (Asante 1994) the alate virginoparae occur initially in November (a few individuals), which are capable of spreading infestation on apples. They then appear again from late January to late April or early May. However, Hely *et al.* (1982), and Thwaite & Bower (1983) believed that the alate females appeared to have no significance other than a reduction of the aphid population on the apple trees, as their fecundity was greatly reduced

(Asante 1994) in comparison with apterous virginoparae. In India alate females appeared from late July (mid-summer) to November (end of autumn) with maximum populations in September (early autumn) to October (mid-autumn) (Gautam & Verma 1983). In the Netherlands *E. lanigerum* population density declined during mid-summer and autumn, probably as a result of the physiological condition of the food plant (Evenhuis 1962).

In South Africa *E. lanigerum* propagates entirely parthenogenetically. During the winter, adults live and feed on the roots. Under the local, milder conditions the parthenogenetic females are also able to overwinter in suitable protected sites on the tree above ground. During the summer winged females are produced which are believed to fly off to start new infestations. The other means of natural dissemination is the dispersal of crawlers by wind, birds and other insects (Georgala 1953, Nel 1983). Carnegie (1963) found that from December onwards, when trees were growing vigorously and new growth was plentiful, there was a rapid increase in aphid population levels. In Zimbabwe numbers reached a peak towards the end of summer, and there was a rapid decline in numbers when colder weather set in and the trees became dormant (Carnegie 1963).

E. lanigerum has a limited ability for between tree dispersal but within tree dispersal of the young nymphs was very high (Asante *et al.* 1993). Throughout the summer, many crawlers dispersed to colonise new growth (Mueller *et al.* 1992). Dumbleton & Jeffreys (1938) in New Zealand found that there was no definite autumn movement to the roots. According to Lohrenz (1911) *E. lanigerum* had little tendency to wander, but moved from the roots when it was hot. Theobald (1920) found an upward movement during early summer and movement down to the roots in the fall, while Froggatt (1903) found that young aphids wandered in an aimless

manner. According to Bodenheimer (1947) in Palestine, the aerial and root colonies developed independently, with little movement between them. Hoyt & Madsen (1960) found that the greatest number of aphids moved up during the summer with a second peak in movement during late October (mid-autumn). This was also found in India (Bhardwaj *et al.* 1995) where the movement up was influenced by temperature and rain.

Locally during spring and summer an endless migration of *E. lanigerum* crawlers from colonies on the roots up into the tree has been reported (Nel 1983). These crawlers started new colonies in the aerial parts. As winter approached a small part of the population moved down to the roots again, where they remained until spring (Nel 1983).

Evenhuis (1958, 1962) found that it was only possible to determine the times at which the aphid populations in the orchard were either 'very high' or 'very low', giving an indication of when the populations were increasing or declining. Asante *et al.* (1993) found a strong relationship between the total number of aphids and the number of colonies, which suggested density-dependant dispersal. This relationship between the total number of *E. lanigerum* and the number of colonies suggested that colony counts could be used to estimate total population size. Counting colonies is economical and less time consuming than counting individuals.

The parasitoid A. *mali* (Hald.) has established in at least 40 countries (De Bach 1964). It was introduced into South African apple orchards from America in 1920 (Lundie 1939). It usually gives satisfactory control where infestation is principally on the twigs, but it fails (e.g. Zimbabwe) where root infestation is important since it does not attack hosts underground (Greathead 1986).

In South Africa no detailed studies have been conducted on the biology of *E. lanigerum* and the published information is sometimes contradictory. Therefore this study was conducted to examine the biology of *E. lanigerum* and of that of its natural enemy, *A. mali,* in apple orchards in the Western Cape Province of South Africa.

4.2 Material and methods

4.2.1. Sites

Numbers of *E. lanigerum* and *A. mali* were monitored on three farms in the Elgin area, namely Molteno (34.10 S 19.3 E), Glenbrae (34.14 S 19.9 E) and Oak Valley (34.9 S 19.2 E). The blocks of apple trees were 2 ha or less. At Molteno aphid and parasitoid numbers were monitored in five blocks for two seasons and thereafter in three blocks. The orchards were planted in 1970 on M193 rootstock. The two blocks in which monitoring was terminated after two seasons consisted only of Granny Smith apple trees. Of the remaining blocks two had Granny Smith trees with alternate rows of Golden Delicious and one had Starking trees with alternate rows of Golden Delicious. Only the Granny Smith and Starking trees were used in this study. On Glenbrae *E. lanigerum* and *A. mali* population levels were monitored in three blocks, two of which were Granny Smith and one was Starking. At the end of the 1992/93 season the Starking block on Glenbrae was removed and at the end of the 1993/94 season the remaining two blocks were also removed, resulting in the termination of sampling on this farm. Information on planting date and rootstock used on Glenbrae were not available. On Oak Valley one 4 ha block consisting of Granny Smith trees were subdivided into two blocks of 2 ha each. This orchard was planted in 1978 on seedling rootstock.

4.2.2 Sampling

i) Sticky stem strips

Masking tape (5 cm wide) painted with a 5 cm long strip of sticky material (Plantex®) was stuck to five evenly spaced trees in each block. On Oak Valley fifteen trees were used. The strips were replaced every second week. During the study (from 1993/94 to 1997/98) stem barriers were used for the control of fruit weevil, *Phlyctinus callosus* Boh. at Molteno and Oak Valley (Barnes *et al.* 1994). At Molteno Protector® Strips were applied to trees from the 1993/94 season and Environbands® from the 1996/97 season. The Protector® Strip is an 8 cm composite of synthetic fibre batting covered with a plastic strip flayed at the bottom edge (Barnes *at al.* 1994). The Environband® consisted of a 5 cm wide plastic strip, factory coated with a non-drying glue, Plantex® (Barnes *et al.* 1994). At Oak Valley an 8 cm wide plastic band was placed around each tree and afterwards painted with Plantex® for fruit weevil control. At these sites one strip of masking tape (5 cm wide) was stuck above and one beneath the fruit weevil barrier. When the masking tape bands were removed the sticky Plantex® was covered with transparent plastic. The bands were then transported to the laboratory where the number of crawlers on each band was counted using a microscope. During the 1991/92 season when only one masking tape band was applied to a tree, crawlers on the top edge were regarded as moving down and those on the lower edge of the band was regarded as moving up. When two bands were applied, crawlers on the top band were regarded as moving down and crawlers on the lower band were regarded as moving up from the roots.

ii) *E. lanigerum* colonies on trees

The number of colonies in the trees was determined by counting them at two weekly intervals on twenty-five evenly spaced trees in each block from the 1991/92 season. Colonies were counted on half of each tree, as it was difficult to manoeuvre through the rows of trees to count the other half. A distinction was made between the colonies with mummies resulting from parasitism by *A. mali* and colonies that were not parasitised. From the 1993/94 season a distinction was also made between colonies in leaf axils and those in wounds. A colony was regarded as one or more feeding aphids, and where heavy infestations caused colonies to become contiguous, each obvious centre of concentration was regarded as a separate colony (Carnegie, 1963). If a branch was covered by aphids, each 2-3 cm of covered wood was regarded as a colony. On Oak Valley 50 evenly spaced trees were monitored in the same way.

(iii) Cylindrical sticky traps

Aerial dispersal of *E. lanigerum* crawlers and *A. mali* was monitored from the 1991/92 season using five cylindrical sticky traps placed between trees in each block. Each trap consisted of a piece of PVC piping 21 cm long and 8 cm in diameter secured vertically to a pole approximately one meter from the ground. Pieces of plastic (overhead projector transparencies) painted with Plantex® were wrapped around the PVC piping and secured using sticky tape. The plastic was replaced every fourth week and the crawlers and *A. mali* adults stuck to it were counted. This was discontinued at the end of the 1992/93 season.

(iv) Yellow sticky traps

From the 1992/93 season the cylindrical traps were replaced by flat, yellow sticky traps, 15cm by 10cm, for monitoring aerial activity of *E. lanigerum* crawlers and *A. mali.* This change was made because handling the yellow traps was less time consuming, they were commercially available and the results produced were similar to those from the cylindrical traps. The traps were initially hung in five evenly spaced trees but from the 1993/94 season they were placed in 12-13 evenly spaced trees. The traps were replaced every fourth week. Transparent plastic was placed on the sticky side of the traps before they were transported to the laboratory where the traps were examined for *E. lanigerum* crawlers and *A. mali* using a microscope.

v) Weather data, spray programmes and soil analysis

Temperature (Table 4.1) and rainfall (Table 4.2) data were obtained from a weather station for the period of the investigation. These weather data were measured at 34.08 S 19.2 E and at an altitude of 305 m in the Elgin district. The chemical sprays which influenced *E. lanigerum* colonies and the dates on which they were applied, are given in Table 4.3 for Molteno and Oak Valley. Spray records from Glenbrae were not available. Soil collected from the orchards on Molteno and Oak Valley were analysed to identify the soil type (Fig. 4.1).

Table 4.1. Average monthly rainfall (mm) in the Grabouw area from 1992 until 1999.

Month	1992	1993	1994	1995	1996	1997	1998	1999
Jan	0.20	0.70	0.70	0.60	0.26	1.21	1.60	0.00
Feb	1.80	2.30	0.60	0.20	2.49	1.11	0.16	0.00
Mar	1.30	0.60	0.60	1.30	2.34	1.50	0.84	
Apr	3.90	9.50	1.90	1.40	1.71	3.21	3.04	
May	4.30	4.40	3.30	4.30	2.68	4.46	7.5	
June	10.60	6.10	11.90	5.50	8.16	6.59	3.78	
July	5.90	8.70	2.80	6.80	5.66	1.17	3.83	
Aug	5.20	3.00	1.90	4.70	3.61	3.40	1.22	
Sept	4.0	0.80	2.50	2.20	5.93	0.43	1.88	
Oct	5.80	0.40	1.80	4.80	5.52	1.55	0.61	
Nov	1.0	1.80	0.40	1.60	4.21	4.87	3.34	
Dec	0.5	1.50	1.90	3.0	3.73	2.10	2.35	

Table 4.2. Average monthly minimum and maximum temperatures in the Grabouw area from 1992 until 1999.

Month	1992		1993		1994		1995		1996		1997		1998		1999	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Jan	14.1	26.1	13.5	26.4	14.7	26.3	14.2	26.3	15.2	26.4	13.8	26.0	12.9	25.8	20.6	27.6
Feb	14.3	25.7	12.6	26.1	14.3	27.0	14.8	27.9	13.4	27.6	14.2	25.5	16.7	28.4	20.5	27.3
Mar	13.6	25.1	13.8	26.9	13.8	25.0	13.2	25.2	11.6	24.9	13.3	25.4	13.2	25.1		
Apr	9.8	20.7	9.8	20.2	11.6	23.9	8.5	21.2	10.1	25.1	9.2	21.9	11.0	24.0		
May	7.8	18.0	8.6	18.2	6.3	19.6	8.7	20.4	7.3	21.7	7.4	21.4	13.5	19.9		
June	6.2	16.6	6.0	17.6	6.8	16.1	6.3	17.2	6.0	18.6	6.1	16.6	12.2	18.2		
July	6.0	16.5	7.3	18.2	4.6	16.9	5.2	14.9	4.4	15.5	5.6	18.5	10.8	17.0		
Aug	5.5	16.8	5.9	18.2	5.4	16.9	6.0	16.7	4.9	16.7	8.2	17.8	11.4	18.6		
Sept	7.6	17.5	7.6	19.9	8.0	19.4	8.3	18.7	7.4	17.4	9.1	21.8	12.4	18.5		
Oct	9.3	19.5	9.6	22.1	9.4	22.6	8.5	18.9	9.2	19.9	10.2	24.1	15.0	22.0		
Nov	10.9	23.1	11.7	24	10.8	22.1	11.3	22.8	9.8	19.4	11.6	22.4	16.0	21.9		
Dec	12.7	23.5	13.2	24.1	12.5	24.9	14.4	24.0	13.0	24.2	13.1	25.5	19.2	25.4		

Table 4.3. Chemicals sprayed for the control of *Eriosoma lanigerum* from the 1992/93 until the 1998/99 season on Molteno and Oak Valley.

Farm	Year	Date	Chemical
Molteno	1992/93	20/8/92	Chlorpyriphos
		21/10/92	Endosulfan
		18/12/92	Vamidothion
	1993/94	6/9/93	Winter oil; chlorpyriphos
		14/10/93	Endosulfan
	1994/95	9/9/94	DNOC & oil; Chlorpyriphos
		10/11/94	Endosulfan
	1995/96	6/9/95	Winter oil; Chlorpyriphos
		21/9/95	Chlorpyriphos
		17/11/95	Endosulfan
	1996/97	10/9/96	DNOC & oil; Chlorpyriphos
		20/9/96	Chlorpyriphos
		3/10/96	Chlorpyriphos
		4/11/96	Endosulfan
		21/3/96	Endosulfan
	1997/98	5/9/97	DNOC & oil; Chlorpyriphos
	1998/99	27/8/98	DNOC & oil; Chlorpyriphos
		26/9/98	Chlorpyriphos
		1/2/99	Chlorpyriphos (Granny Smith only)
Oak Valley	1992/93	8/9/92	DNOC & oil; Prothiofos
		15/9/92	Prothiofos
		5/10/92	Endosulfan
		10/11/92	Phosalone
		16/11/92	Phosalone
		22/12/92	Endosulfan
		30/12/92	Phosalone
		12/1/93	Phosalone
		20/1/93	Endosulfan
		31/3/93	Endosulfan
	1993/94	25/8/93	DNOC & oil; Prothiofos
		6/9/93	Chlorpyriphos
		4/10/93	Endosulfan
		1/11/93	Endosulfan
		10/1/94	Endosulfan
	1994/95	30/8/94	DNOC & oil; Chlorpyriphos
		3/10/94	Endosulfan
		27/10/94	Endosulfan
		22/11/94	Endosulfan
		1/2/95	Endosulfan
		10/3/95	Endosulfan
	1995/96	5/9/95	DNOC & oil; Chlorpyriphos

Table 4.3. (Continued)

		19/9/95	Prothiofos
		6/11/95	Endosulfan
		3/1/96	Chlorpyriphos
		24/4/96	Chlorpyriphos
	1996/97	4/9/96	DNOC & oil; Chlorpyriphos
		26/9/96	Prothiofos
		5/11/96	Endosulfan
		12/11/96	Endosulfan
	1997/98	28/8/97	DNOC & oil; Prothiofos
		7/10/97	Endosulfan
		21/1/98	Endosulfan
	1998/99	25/8/98	DNOC & oil; Chlorpyriphos
		16/9/98	Prothiofos
		8/12/98	Endosulfan
		26/1/99	Chlorpyriphos

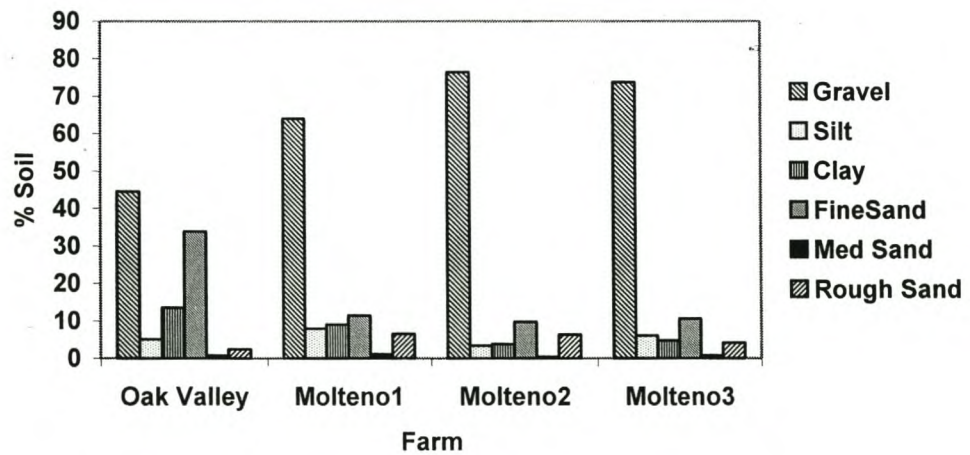

Fig. 4.1. Percentage of each component of the soil from Oak Valley and the three blocks on Molteno.

4.3. Results

4.3.3. Sticky stem bands

The migration of *E. lanigerum* crawlers from the roots up into the apple trees followed a similar pattern during most seasons (Figs. 4.1.1 - 4.1.5). However, the time

at which upward movement started and peaked differed from season to season. *E. lanigerum* crawlers were recorded on the strips throughout the year on all the farms. However, the numbers were very low during the winter.

On Glenbrae upward movement had already started when the study began in November 1991 (Fig. 4.1.1A). During the subsequent seasons it started from October 1993 (Fig. 4.1.1C) and from mid-November 1992 (Fig. 4.1.1B). Peak upward migration was recorded from mid- November to early December during these seasons respectively (Fig. 4.1.1) with the highest crawler numbers recorded during the 1991/92 season at more than 50 crawlers per band (Fig. 4.1.1A).

On Molteno upward migration usually started during September (Fig. 4.1.2B) or October (Figs. 4.1.2 C, D and 4.1.3 A, C and D). A slight decrease in numbers of crawlers was recorded during October 1992 (Fig. 4.1.2B) before numbers increased again. Upward migration started later during the 1996/97 season (end December) (Fig. 4.1.3B) than in the other seasons.

Peak numbers moving up were usually recorded soon after upward migration started (Figs. 4.1.2 and 4.1.3) except during the 1995/96 season when a second and higher peak was recorded during February 1996 (Fig. 4.1.3A) and a third peak during March 1996. Upward migration of *E. lanigerum* crawlers was highest during the 1996/97 (Fig. 4.1.3B) and 1997/98 (Fig. 4.1.3C) seasons. During these two seasons the peak in this upward migration was followed by a dramatic decrease in the number of crawlers moving up the trunks. The lowest number of crawlers moving up was recorded during the 1998/99 season (Fig. 4.1.3D).

On Oak Valley upward migration of *E. lanigerum* crawlers usually started when the winter rainfall ceased (Table 4.1). More crawlers were usually recorded

from the end of August (Figs. 4.1.5A and B) or September (Figs. 4.1.4 C, D and 4.1.5 C) than during the winter months. However, crawler movement also started as late as October (Fig. 4.1.4B) or November (Fig. 4.1.4A) in the 1993/94 and 1992/93 seasons respectively.

Peak numbers of crawlers moving up were usually recorded during November (Figs. 4.1.4 and 4.1.5). However, during the 1997/98 season the highest numbers were recorded during October (Fig. 4.1.5B). During the 1996/97 season a second and higher peak in December followed the first peak in November (Fig. 4.1.5A).

On Oak Valley a second and third peak in upward migration was also recorded during some seasons. This was clearly visible during early February and May 1994 (Fig. 4.1.4B) and the end of January and April 1995 (Fig. 4.1.4D). Crawler numbers on the bands were usually high at times when there were large numbers of colonies in the aerial parts of the trees (Figs. 4.1.4 B and D).

Crawlers were also recorded moving down when high numbers of crawlers were recorded moving up, except for the 1993/94 season (Fig. 4.1.4B). During the 1993/94 (Fig. 4.1.4B) and 1994/95 (Fig. 4.1.4C) seasons the highest number of crawlers were recorded moving up and the lowest were recorded during the 1992/93 (Fig. 4.1.4A) and 1996/97 (Fig. 4.1.5A) seasons.

4.3.2 *E. lanigerum* colonies on trees

The number of *E. lanigerum* colonies in the aerial parts of the apple trees varied within seasons and between seasons on Glenbrae (Fig. 4.2.1), Molteno (Figs. 4.2.2 and 4.2.3) and Oak Valley (Figs. 4.2.4 and 4.2.5). *E. lanigerum* colonies were usually visible during the winter but their numbers declined when the trees were

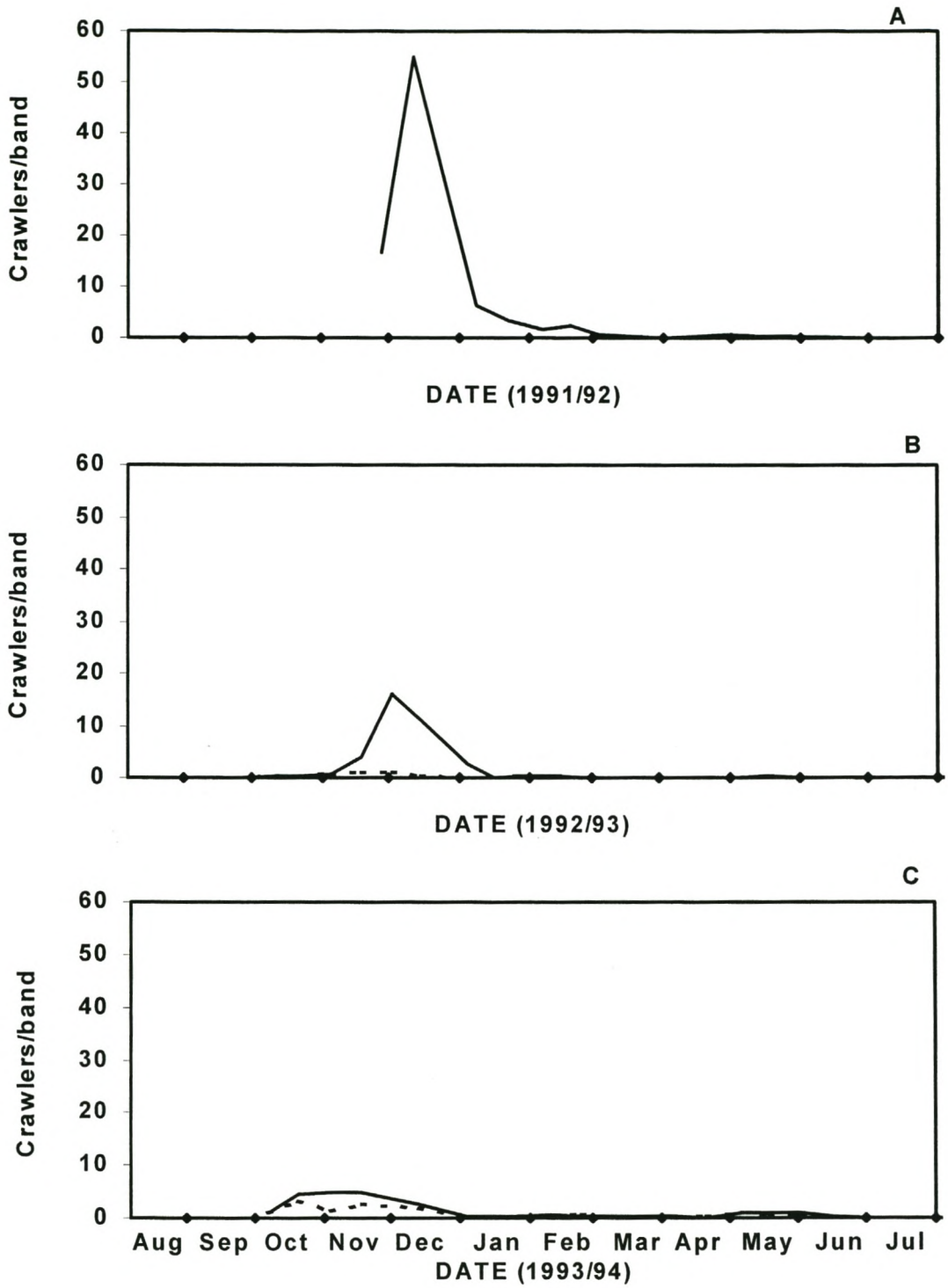

Fig. 4.1.1. Average number of *Eriosoma lanigerum* crawlers moving up (solid line) and down (broken line) apple trees on Glenbrae during the 1991/92 (A), 1992/93 (B) and 1993/94 (C) seasons.

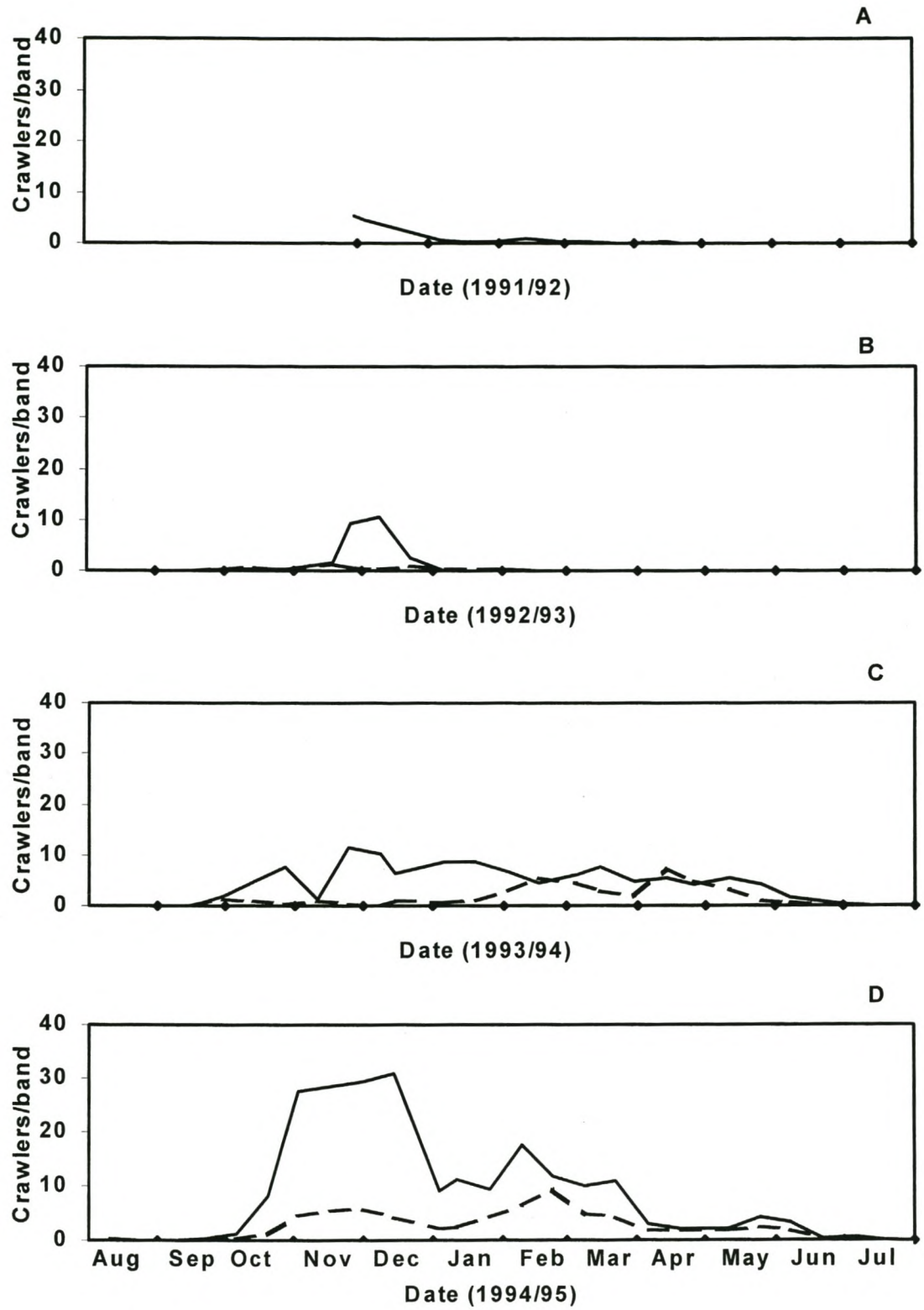

Fig. 4.1.2. Average number of *Eriosoma lanigerum* crawlers moving up (solid line) and down (broken line) apple trees on Molteno during the 1991/92 (A), 1992/93 (B), 1993/94 (C) and 1994/95 (D) seasons.

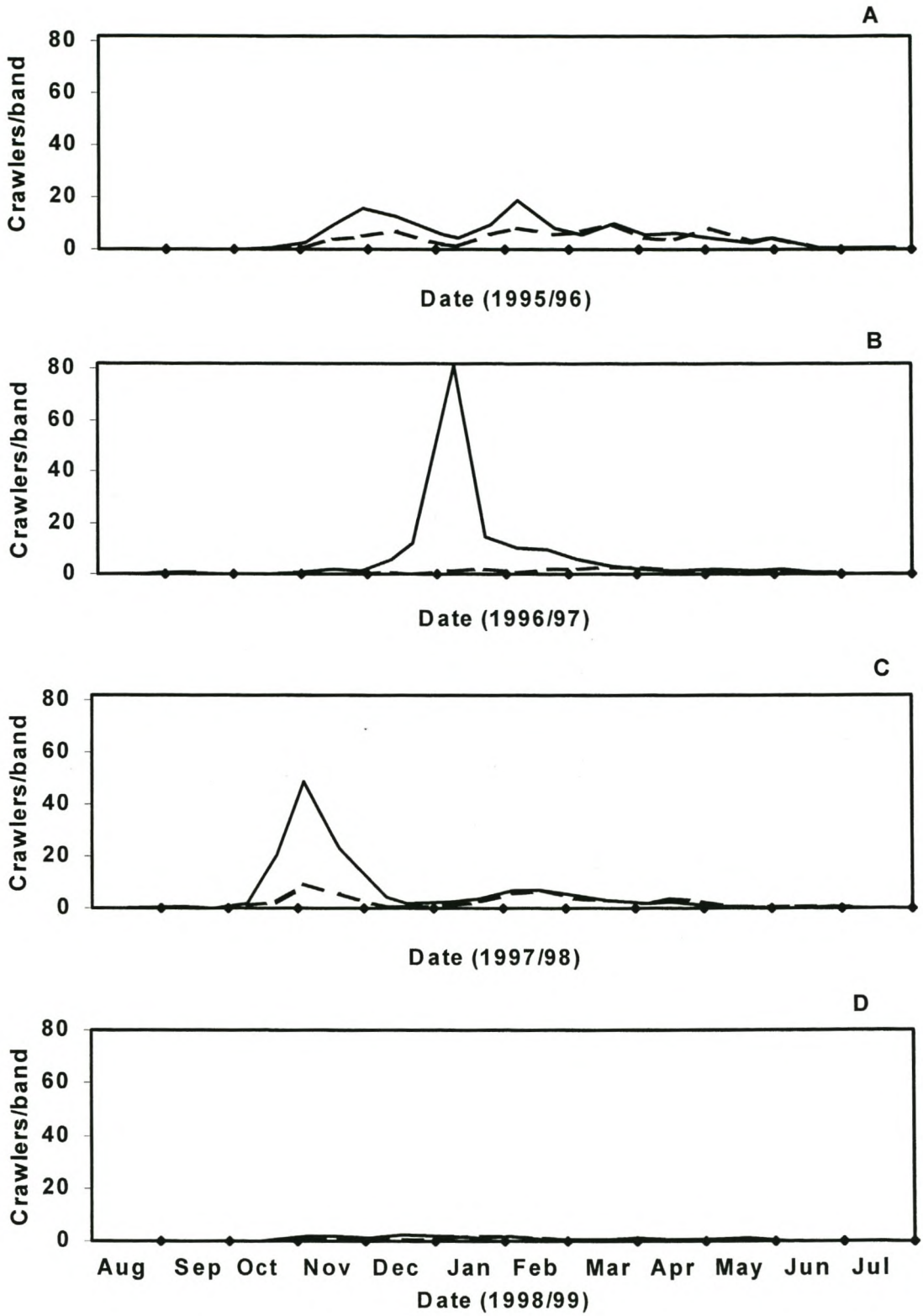

Fig. 4.1.3. Average number of *Eriosoma lanigerum* crawlers moving up (solid line) and down (broken line) apple trees on Molteno during the 1995/96 (A), 1996/97 (B), 1997/98 (C) and 1998/99 (D) seasons.

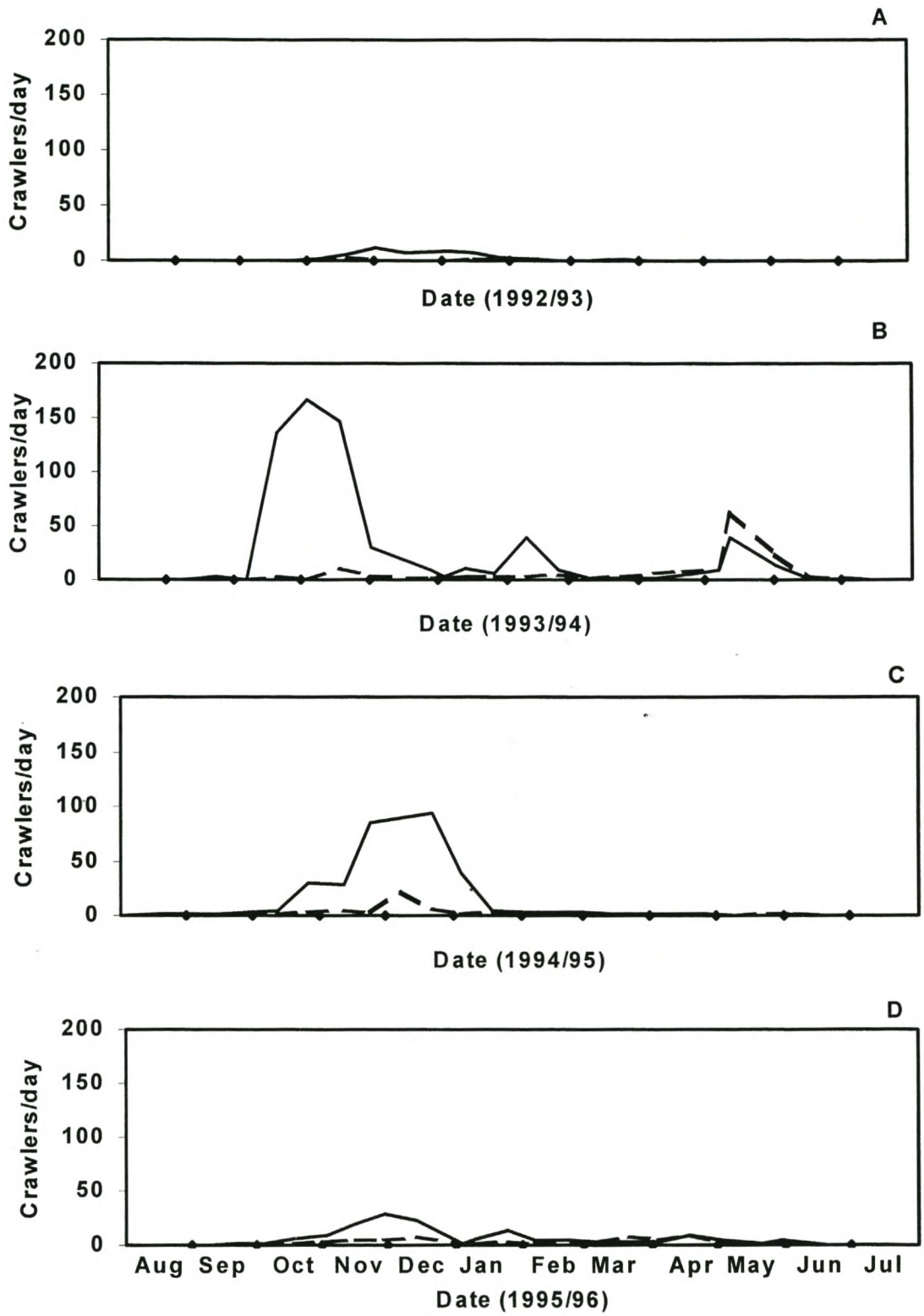

Fig. 4.1.4. Average number of *Eriosoma lanigerum* crawlers moving up (solid line) and down (broken line) the apple trees on Oak Valley during the 1992/93 (A), 1993/94 (B), 1994/95 (C) and 1995/96 (D) seasons.

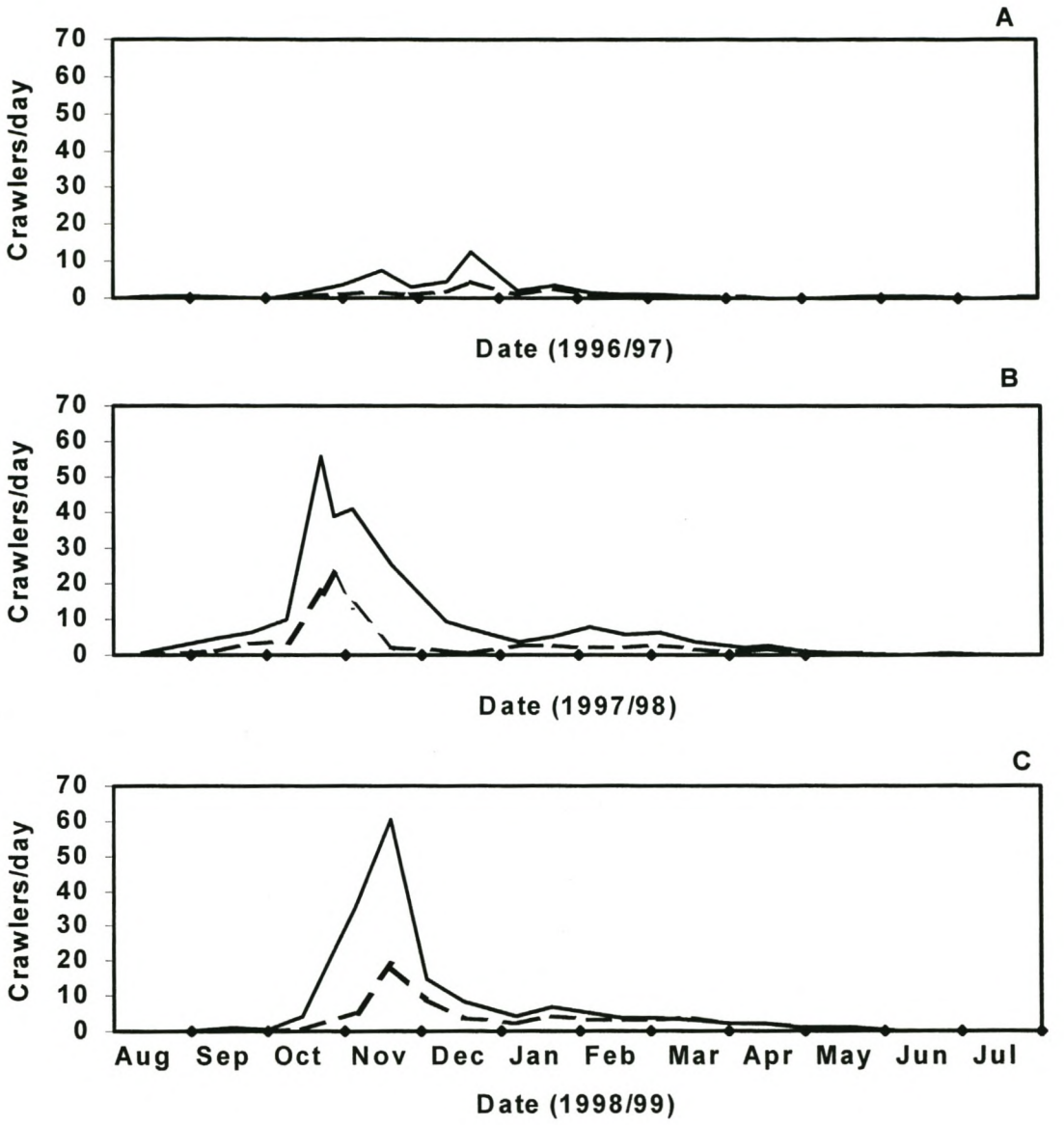

Fig. 4.1.5. Average number of *Eriosoma lanigerum* crawlers moving up (solid line) and down (broken line) apple trees on Oak Valley during the 1996/97 (A), 1997/98 (B) and 1998/99 (C) seasons.

pruned, and mostly disappeared when chemicals were applied to combat delayed foliation (DNOC & oil, winter oil), *Quadraspidiotus perniciosus* (Comstock) and *Pseudococcus* pests (chlorpyrifos; prothiofos) as these two products are registered for *E. lanigerum* control (Nel *et al.* 1999).

The first *E. lanigerum* colonies were usually recorded during December or early January, which was a few weeks after the crawlers moved up into the trees. This was the case on Glenbrae where the first new colonies were recorded from early to mid-December during all the seasons (Fig. 4.2.1). From February during each season *E. lanigerum* colonies started to increase rapidly and reached maximum numbers from early to mid-March (Fig. 4.2.1) followed by a decline in numbers soon afterwards. A second smaller peak in the number of colonies was recorded at the end of May 1992 (Fig. 4.2.1A), and also during May 1994 (Fig. 4.2.1C). This late peak was not obvious during the 1992/93 (Fig. 4.2.1B) season.

The first parasitised colonies on Glenbrae were recorded from mid- to end-February (Fig. 4.2.1). At this time winged female aphids were also recorded in the apple trees. By the end of March of each season all the colonies contained mummified aphids as a result of parasitism by *A. mali*. When the colder weather set in more unparasitised colonies were again recorded. This was apparent during July 1992 (Fig. 4.2.1A) and again in May 1994 (Fig. 4.2.1C) on Glenbrae.

The first new *E. lanigerum* colonies on Molteno appeared in low numbers from November (Figs. 4.2.2D and 4.2.3 A, C) or December (Figs. 4.2.2A, B and C). However, during the 1996/97 season the first colonies were only found from the end of January (Fig. 4.2.3B). During the 1998/99 season the first unparasitised colonies were also recorded from January (Fig. 4.2.3D). Colony numbers remained low until the end of January or February when they increased rapidly (Figs. 4.2.2 and 4.2.3) and

usually reached a maximum during March (Figs. 4.2.2 A, B, C, D and 4.2.3 B, D) or early April (Fig.4.2.3C). The only exception was during the 1995/96 season when maximum numbers of colonies were recorded at the end of autumn (Fig. 4.2.3A). This was also the season during which the highest *E. lanigerum* populations were recorded, with an average of 80.17 colonies per half tree.

The first parasitised colonies on Molteno were usually recorded from the end of January or during February (Figs. 4.2.2 and 4.2.3) except for the 1994/95 season (Fig. 4.2.2D) when a few parasitised colonies were recorded in December 1994. From the end of March or April most or all of the colonies contained parasitised mummies. The number of colonies per half tree usually declined after March or when a chemical spray was applied (Table 4.3) for the control of *E. lanigerum*. After the endosulfan spray applied during March 1997 (Fig. 4.2.3B) and chlorpyrifos during February 1999 (Fig. 4.2.3D) the colony numbers declined but increased slightly again from the middle of the following month during both seasons. Colonies of *E. lanigerum* were always recorded during the winter on Molteno (Figs. 4.2.2 and 4.2.3) until the trees were pruned and sprays applied (Table 4.3) at the end of winter.

On Oak Valley chemical sprays were applied more frequently for the control of *E. lanigerum* colonies (Table 4.3). Therefore, the basic pattern of development of colony numbers and that of its parasitoid was more variable than on the other two sites. Overwintering colonies were removed by the chemicals sprayed at the end of winter or spring (Table 4.3) (Figs. 4.2.4 and 4.2.5). New unparasitised colonies were usually found in very low numbers during December (Figs. 4.2.4 A, D and 4.2.5C). However, a few colonies were found as early as mid-November during the 1997/98

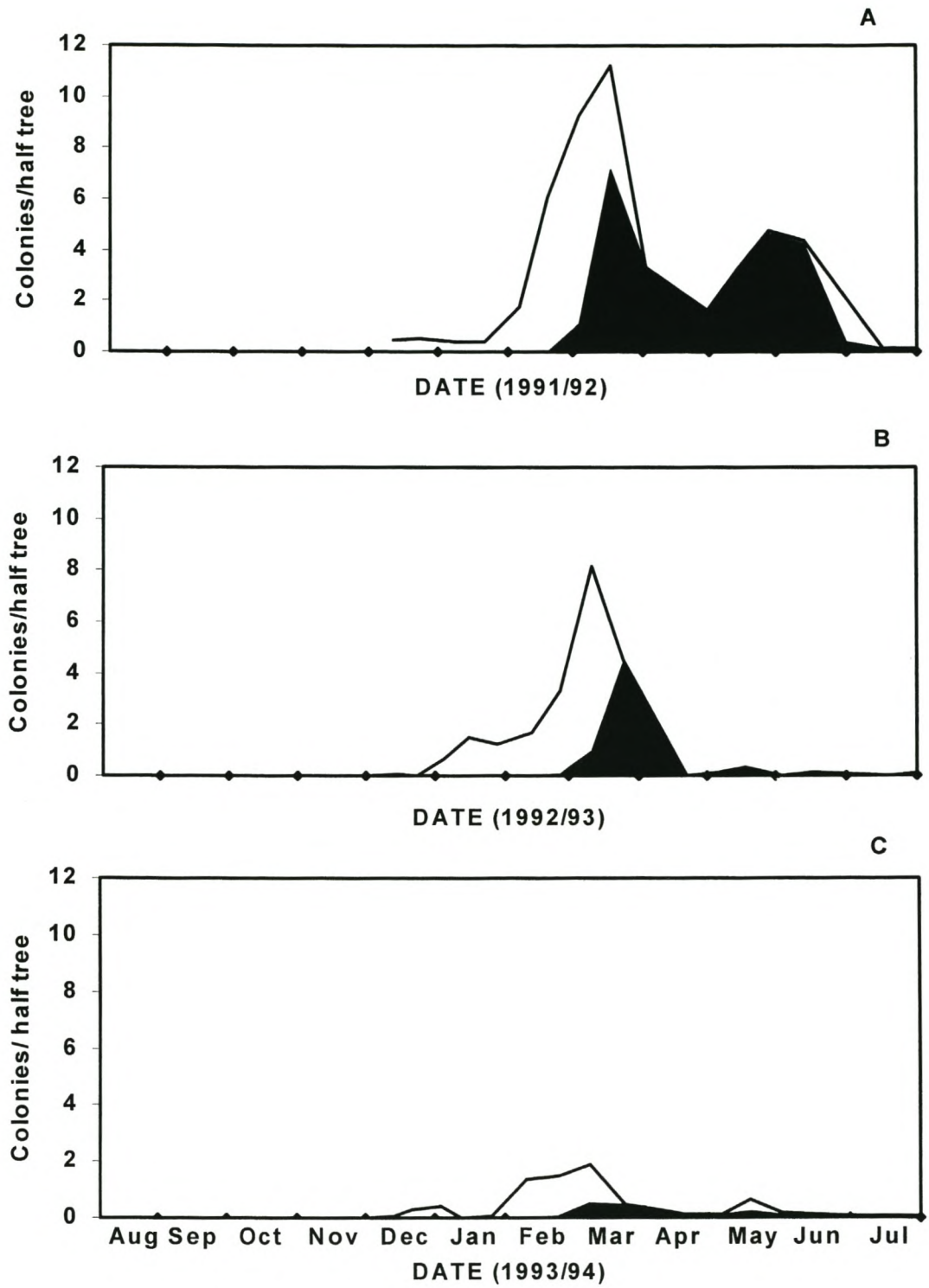

Fig. 4.2.1. Total (parasitised and unparasitised) *Eriosoma lanigerum* colonies per half tree (clear area) and *E. lanigerum* colonies per half tree parasitised by *Aphelinus mali* (shaded area) on Glenbrae during the 1991/92 (A), 1992/93 (B) and 1993/94 (C) seasons.

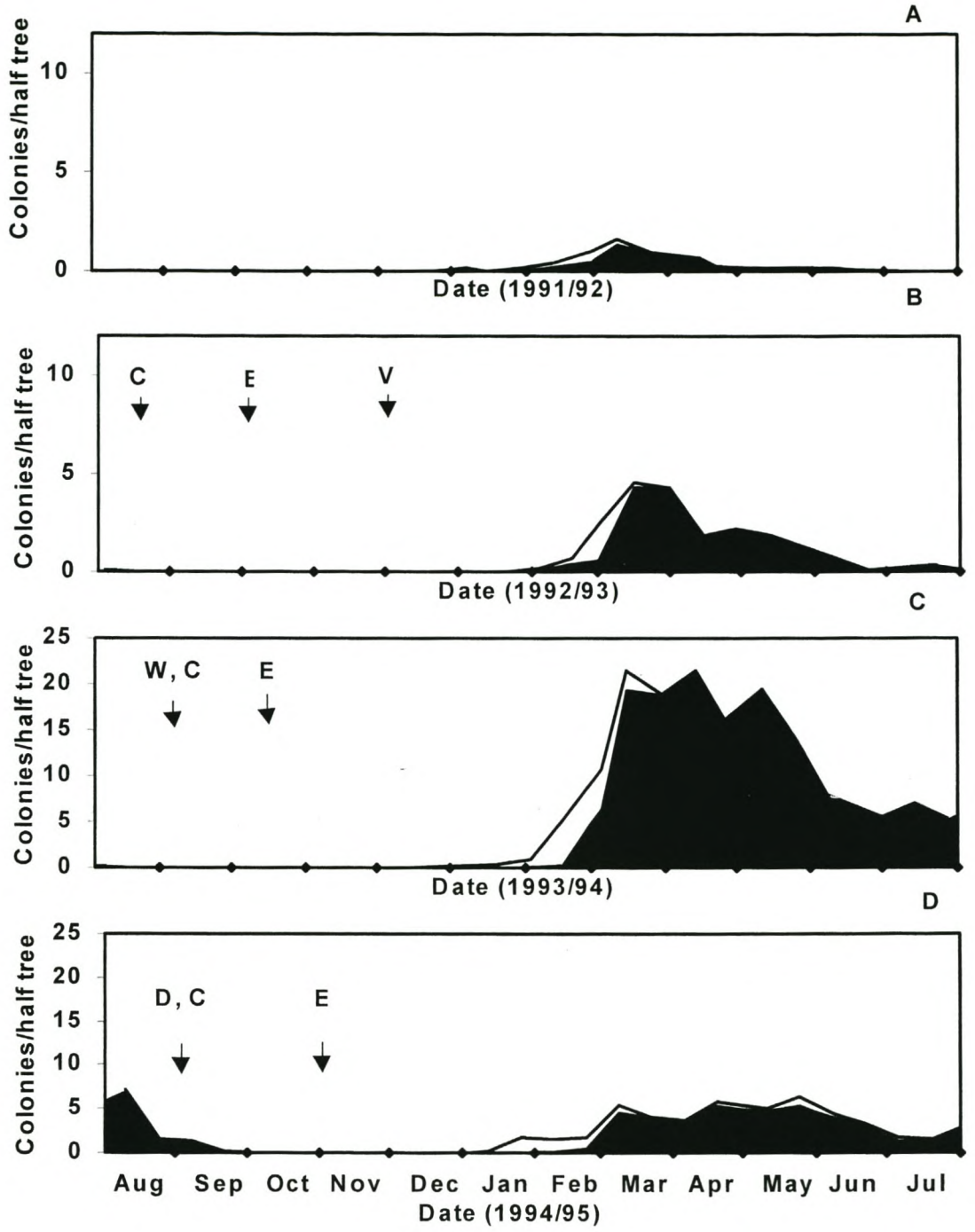

Fig. 4.2.2. Total (parasitised and unparasitised) *Eriosoma lanigerum* colonies per half tree (clear area) and colonies per half tree parasitised by *Aphelinus mali* (shaded area) on Molteno for the 1991/92 (A), 1992/93 (B), 1993/94 (C) and 1994/95 (D) seasons. Chemical sprays applied for *E. lanigerum* control: C=chlorpyrifos, E=endosulfan, V=vamidothion, W=winter oil, D=DNOC & oil.

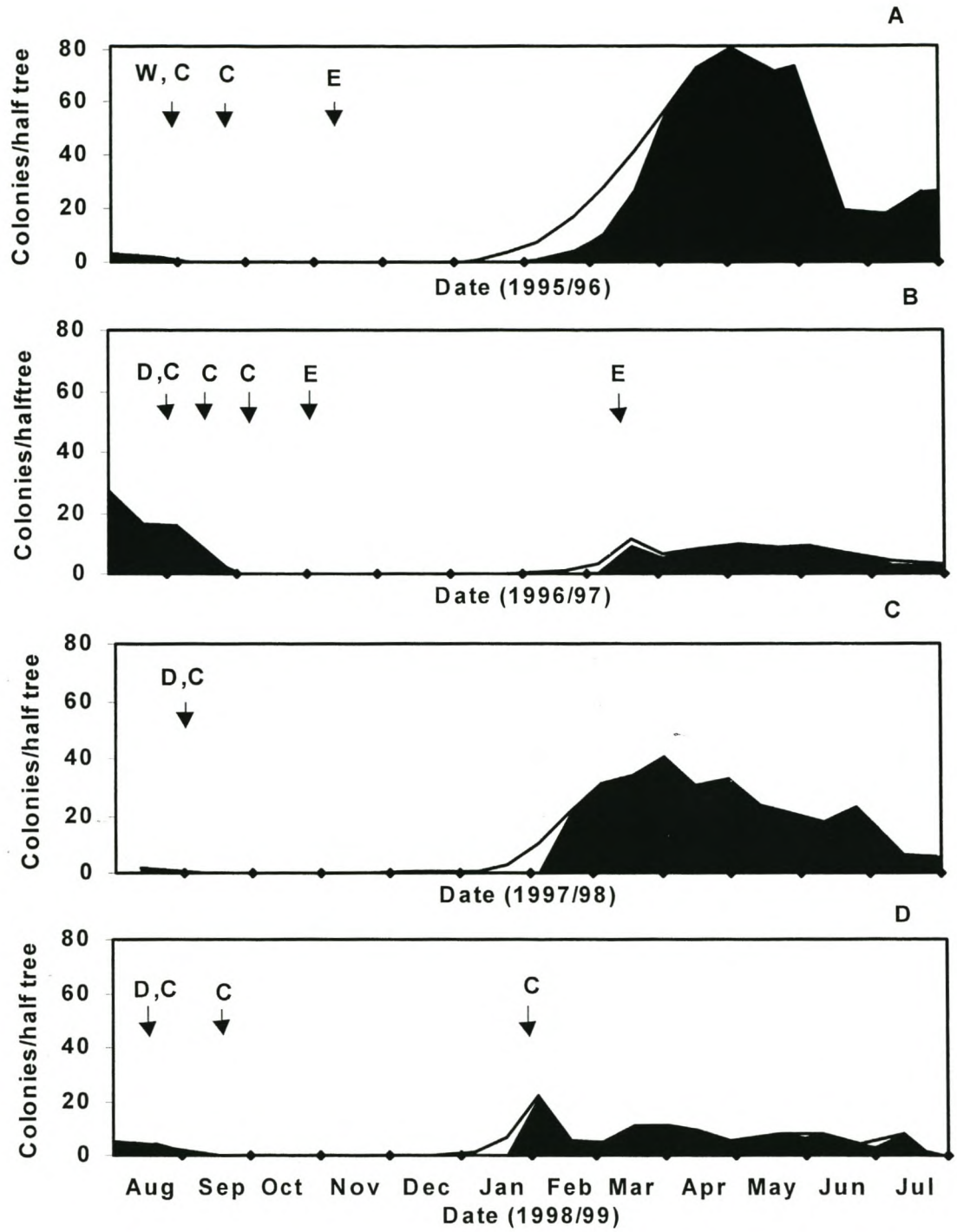

Fig. 4.2.3. Total (parasitised and unparasitised) *Eriosoma lanigerum* colonies per half tree (clear area) and colonies per tree parasitised by *Aphelinus mali* (shaded area) on Molteno for the 1995/96 (A), 1996/97 (B), 1997/98 (C) and 1998/99 (D) seasons. Chemical sprays applied for *E. lanigerum* control: W=winter oil, C=chlorpyrifos, E=endosulfan, D=DNOC & oil.

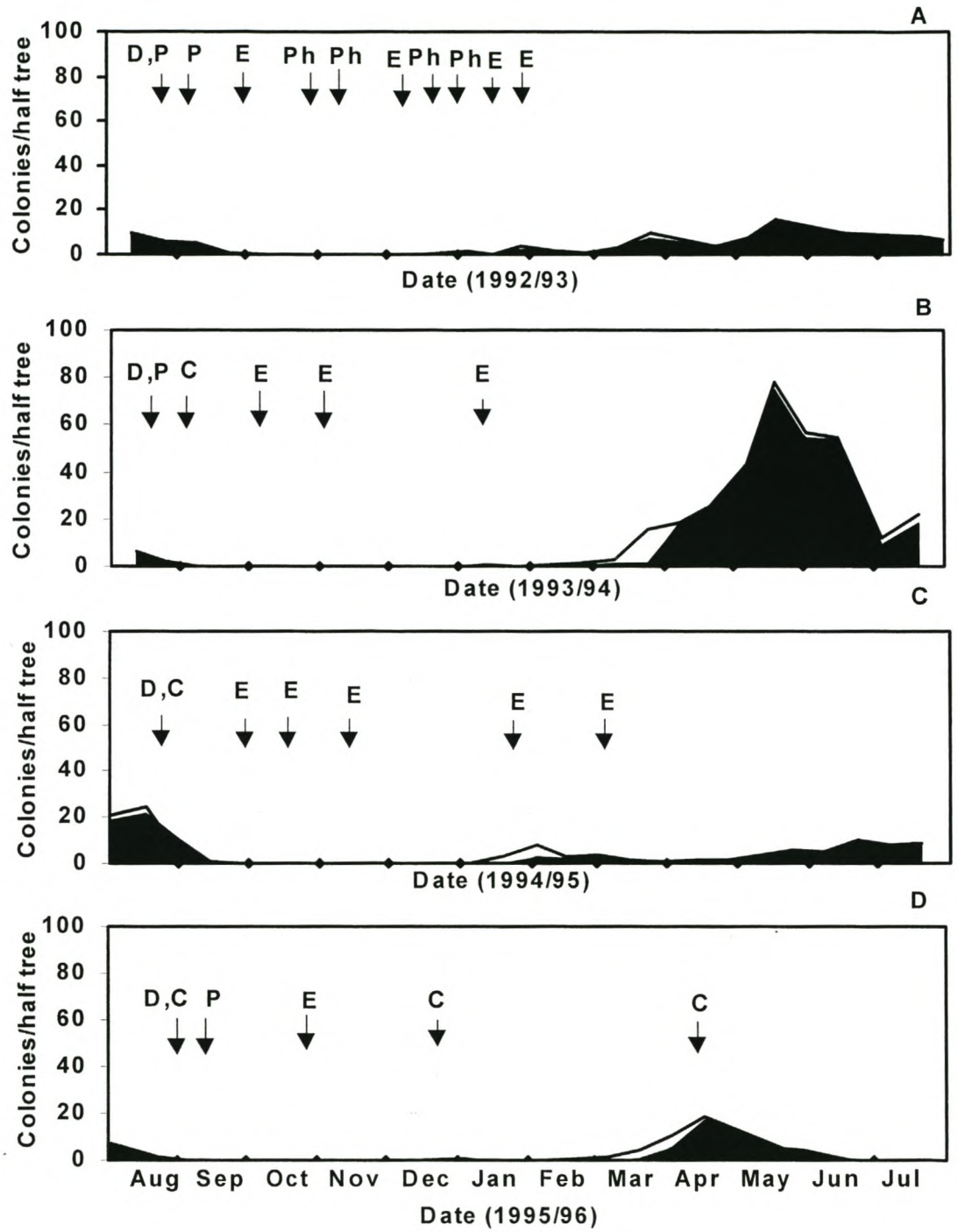

Fig. 4.2.4. Total (parasitised and unparasitised) *Eriosoma lanigerum* colonies per half tree (clear area) and colonies per half tree parasitised by *Aphelinus mali* (shaded area) on Oak Valley during the 1992/93 (A), 1993/94 (B), 1994/95 (C) and 1995/96 (D) seasons. Chemical sprays applied for *E. lanigerum* control: D=DNOC & oil, P=prothiofos, E=endosulfan, Ph=phosalone, C=chlorpyrifos.

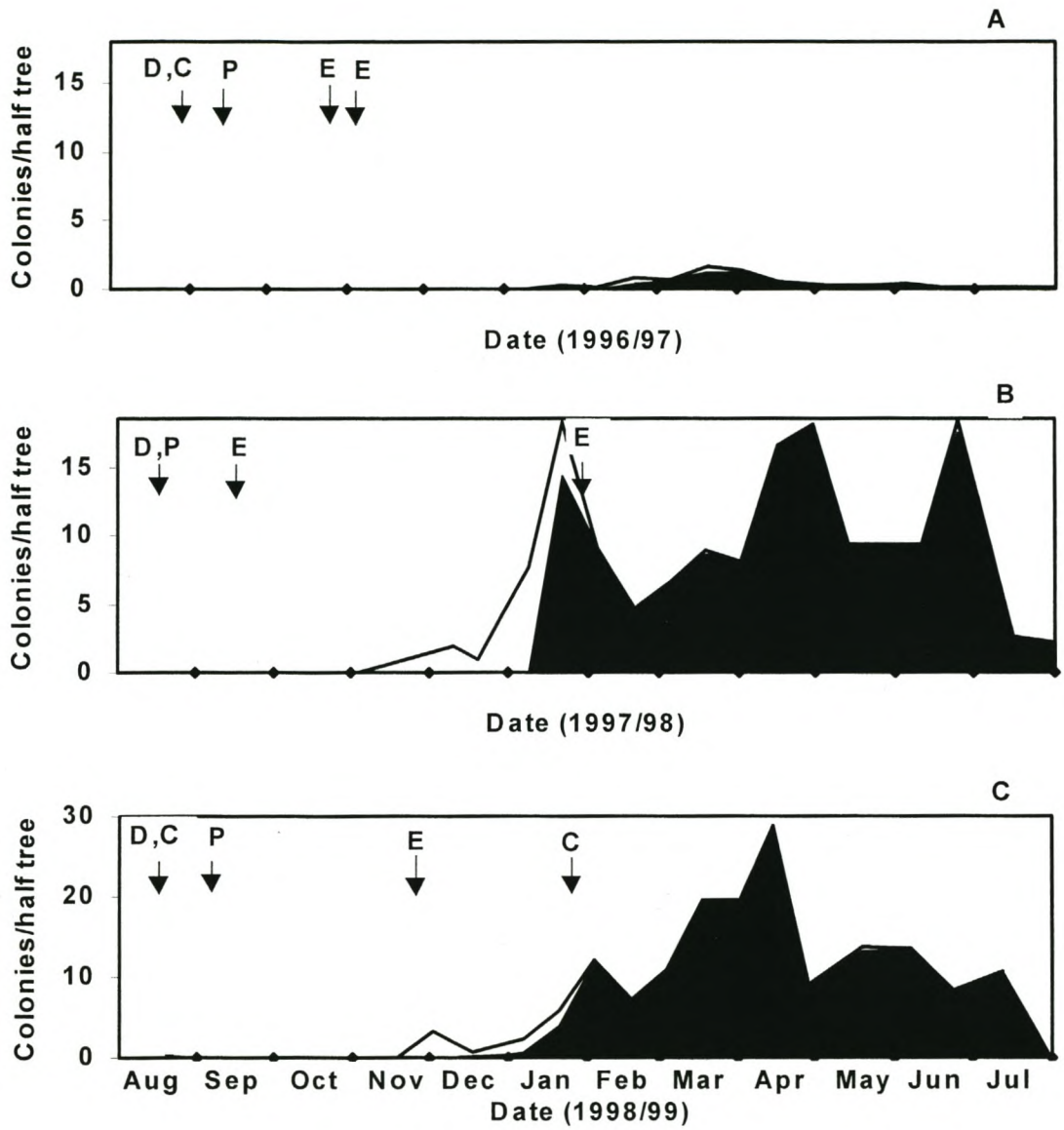

Fig. 4.2.5. Total (parasitised and unparasitised) *Eriosoma lanigerum* colonies per half tree (clear area) and *E. lanigerum* colonies per half tree parasitised by *Aphelinus mali* (shaded area) on Oak Valley during the 1996/97 (A), 1997/98 (B) and 1998/99 (C) seasons. Chemical sprays applied for *E. lanigerum* control: D=DNOC & oil, C=chlorpyrifos, P=prothiofos, E=endosulfan.

season (Fig. 4.2.5B) or as late as January (Figs. 4.2.4B, C and 4.2.5A). During the 1996/97 season the first colonies were recorded very late (Fig. 4.2.5A) and the number of colonies recorded during this season was very low.

The maximum number of colonies on Oak Valley was recorded from January until July depending on the time at which chemical sprays were applied. The first parasitised colonies were usually recorded during January (Fig. 4.2.4B) or February (Figs. 4.2.4 C, D and 4.2.5A). However, during the 1992/93 season low numbers of parasitised colonies were recorded early in December whereafter an endosulfan spray was applied. Parasitised colonies were again found at the end of January (Fig. 4.2.4A). Low numbers of parasitised colonies were also found during November 1997 and again from January 1998 (Fig. 4.2.5B). During the 1998/99 season the first parasitised colonies were recorded from December but only increased from mid-January 1999 (Fig. 4.2.5C). Although most colonies were parasitised from the end of January to February (Figs. 4.2.4 and 4.2.5) most of the colonies were only parasitised during April in seasons when colonies made a late appearance in the trees (Figs. 4.2.4 B and D).

4.3.3 Cylindrical sticky traps

The cylindrical traps monitored the aerial movement of *E. lanigerum* crawlers as well as the activity of adult *A. mali*. Crawler numbers increased on the cylindrical traps during spring and late summer (Figs. 4.3.1, 4.3.2 and 4.3.3). The spring peak coincided with the time when the upward migration from the roots into the trees took place and the late summer peak with peak colony numbers in the aerial parts of the trees. *E. lanigerum* crawlers were recorded from spring until the end of winter when

winter sprays were applied for the control of other pests. *A. mali* was recorded on the cylindrical traps from mid-summer, through the winter until chemicals were applied at the end of winter. Peak numbers were recorded during autumn when the number of parasitised colonies in the trees was high.

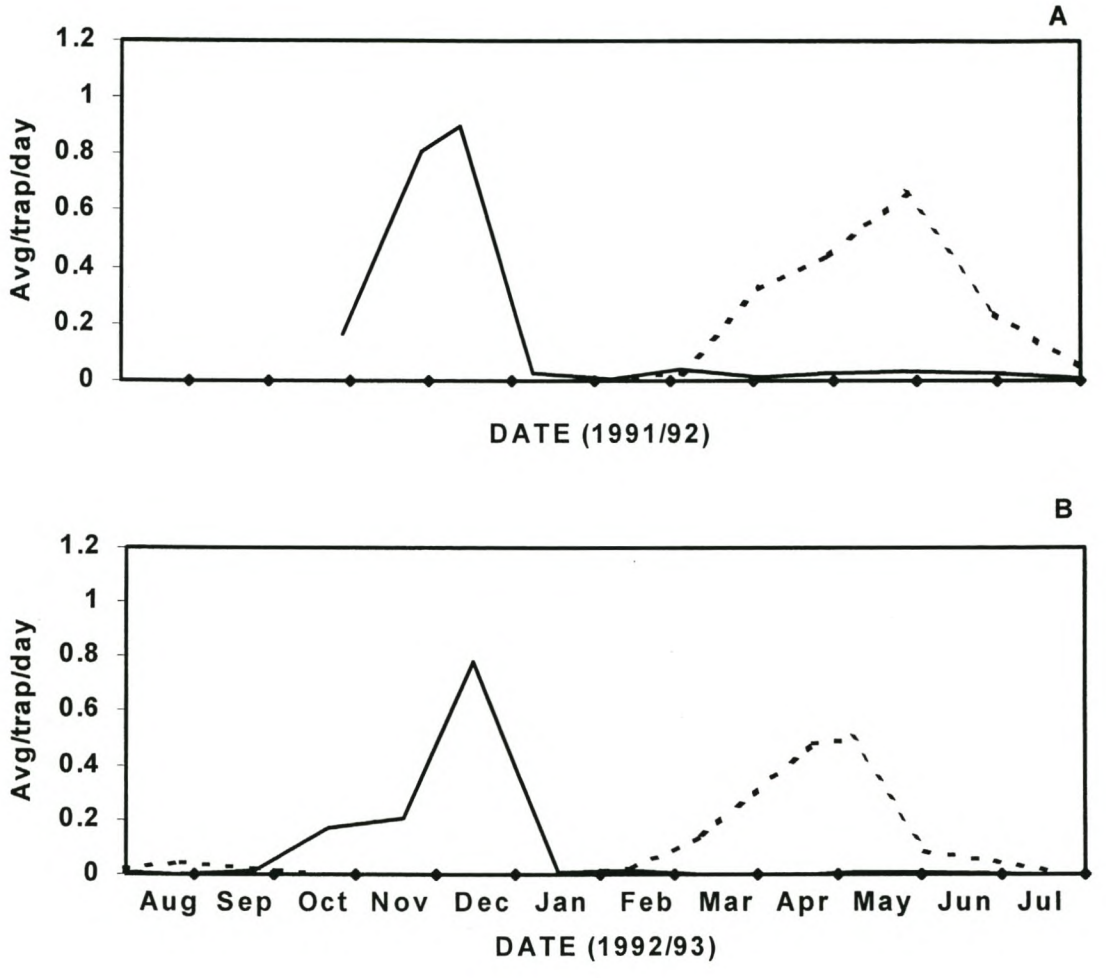

Fig. 4.3.1. Average number (Avg.) of *Eriosoma lanigerum* crawlers (solid line) and *Aphelinus mali* adults (broken line) on the cylindrical sticky traps on Glenbrae during the 1991/92 (A) and 1992/93 (B) seasons.

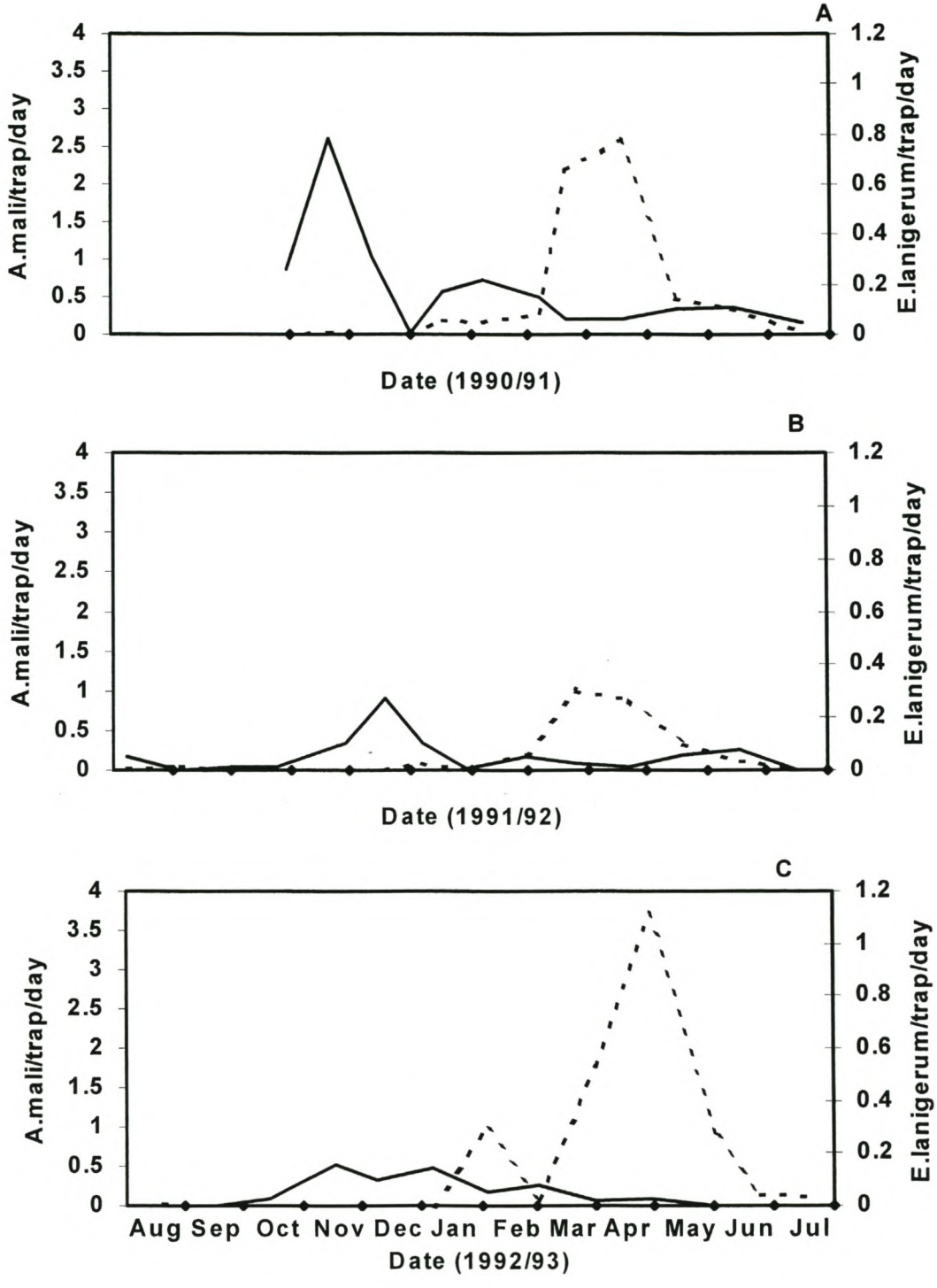

Fig. 4.3.2. Average number of *Eriosoma lanigerum* crawlers (solid line) and *Aphelinus mali* adults (broken line) on the cylindrical traps on Molteno during the 1990/91 (A), 1991/92 (B) and 1992/93 (C) seasons.

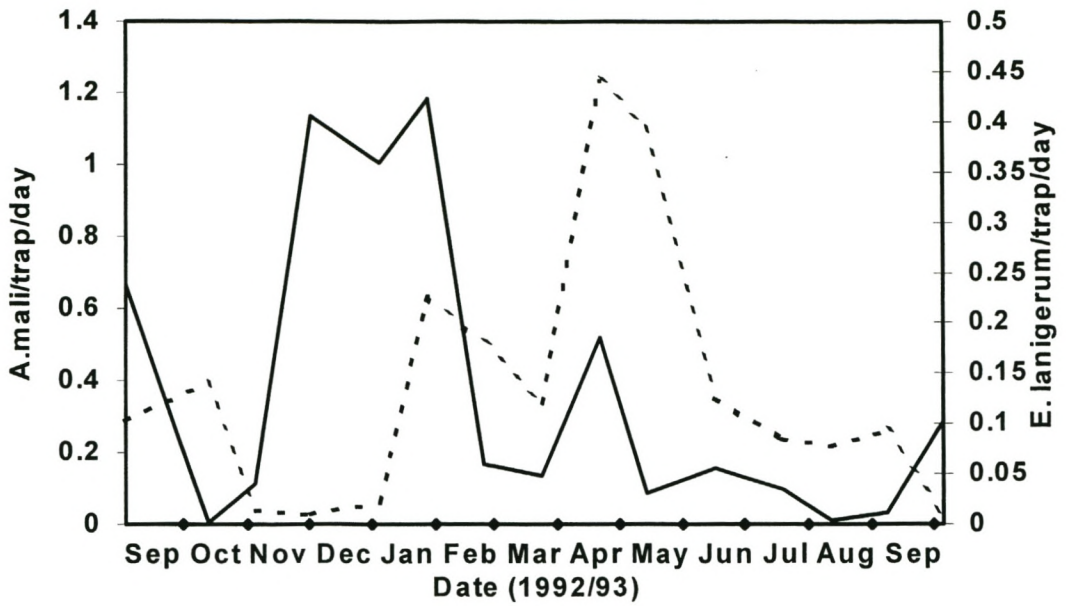

Fig. 4.3.3. Average number of *Eriosoma lanigerum* crawlers (solid line) and *Aphelinus mali* adults (broken line) on the cylindrical traps on Oak Valley during the 1992/93 season.

On Glenbrae *E. lanigerum* crawler numbers on the cylindrical traps peaked from the end of spring until the beginning of summer during both seasons (Fig. 4.3.1A and B) after which low numbers of crawlers were recorded for the rest of the season. *A. mali* were found on the cylindrical traps from February during both seasons (Fig. 4.3.1A and B), with peak numbers recorded during May 1992 and the end of April 1993. Parasitoids were recorded on the cylindrical traps throughout the winter until September 1992 and the end of August 1993 (Fig. 4.3.1B).

The same trend occurred on Molteno with the highest numbers of *E. lanigerum* crawlers recorded at the end of spring (Fig. 4.3.2). *A. mali* numbers were highest from March until April after which their numbers declined rapidly and stayed low throughout the winter (Fig. 4.3.2).

On Oak Valley, the cylindrical traps were used for only one season. *E. lanigerum* crawlers were recorded in high numbers during September 1992 (Fig. 4.3.3) when the investigation was started and their numbers declined during October. The following season the first peak in crawler numbers was recorded during December 1992 and January 1993. Another peak was apparent during April 1993 after which crawler numbers remained low throughout the winter. A few *A. mali* were recorded during September 1992 but their numbers only started to increase from January 1993 (Fig. 4.3.3). Parasitoid numbers declined during February 1993 but increased again after March to reach peak numbers during April 1993.

4.3.4 Yellow sticky traps

E. lanigerum crawlers usually appeared on the yellow traps in low numbers during spring and/or early summer on Glenbrae (Fig. 4.4.1), Molteno (Figs. 4.4.2 and 4.4.3) and Oak Valley (Figs. 4.4.4 and 4.4.5). On Glenbrae peak numbers of *E.*

lanigerum crawlers were recorded during spring (Fig. 4.4.1) with a second smaller peak during the summer of the 1993/94 season (Fig. 4.4.1B). However, on the other two sites most crawlers were usually recorded at the end of summer (Figs. 4.4.2, 4.4.3, 4.4.4 and 4.4.5) when numbers of *E. lanigerum* colonies were higher in the trees. On Glenbrae the average number of crawlers that was recorded on the yellow traps was very low, with a maximum of only one per trap per day at the end of October 1993 (Fig. 4.4.1B). This was also the case on Molteno except during the 1995/96 (Fig. 4.4.2D) and 1997/98 seasons (Fig. 4.4.3B) when peak numbers were recorded during April and March respectively. Crawler numbers declined soon thereafter.

On Oak Valley crawlers were recorded on the yellow sticky traps from spring with the first peak usually occurring during the end of spring or during summer (Fig. 4.4.4 and 4.4.5). Low numbers of crawlers were recorded during the 1994/95 (Fig. 4.4.4C) and 1996/97 seasons (Fig. 4.4.5A), while the highest number of crawlers was recorded during the 1993/94 (Fig. 4.4.4B) and 1998/99 (Fig. 4.4.5C) seasons.

A. mali adults were usually recorded throughout the season (Figs. 4.4.1- 4.4.5.) except during early spring after the chemical sprays (Table 4.2) were applied at the end of winter. Few parasitoids were recorded on the yellow traps early during summer on all the farms and their numbers usually only started to increase from the end of summer, reaching peak population levels soon afterwards during autumn. On Glenbrae parasitoid numbers never reached more than 0.6 per trap per day (Fig. 4.4.1) and the time when parasitoids were recorded did not coincide with the time *E. lanigerum* crawlers were found on the yellow sticky traps.

On Molteno the number of parasitoids was usually much higher than that of the crawlers (Figs. 4.4.2 and 4.4.3), except for the 1995/96 season (Fig. 4.4.2D).

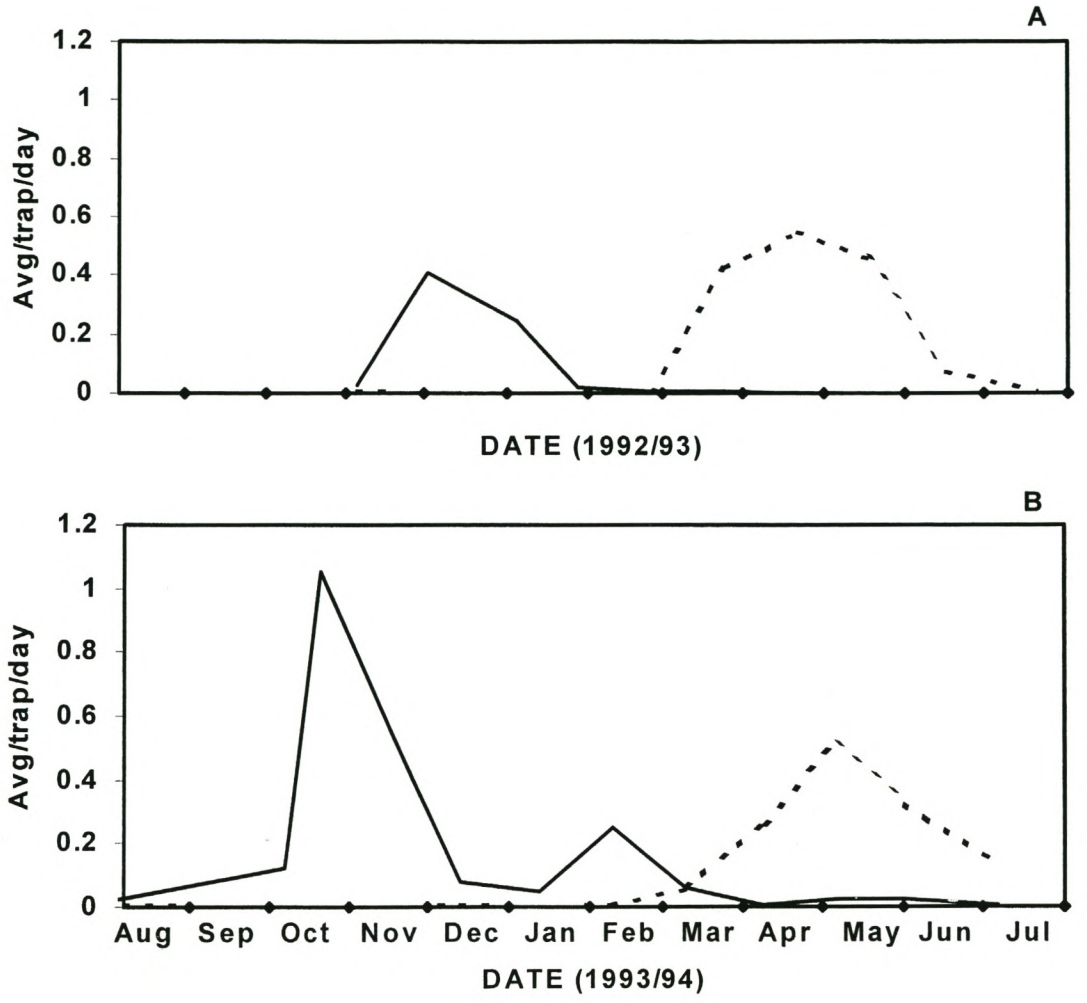

Fig. 4.4.1. Average number (Avg.) of *Eriosoma lanigerum* crawlers (solid line) and *Aphelinus mali* adults (broken line) on the yellow sticky traps on Glenbrae during the 1992/93 (A) and 1993/94 (B) seasons.

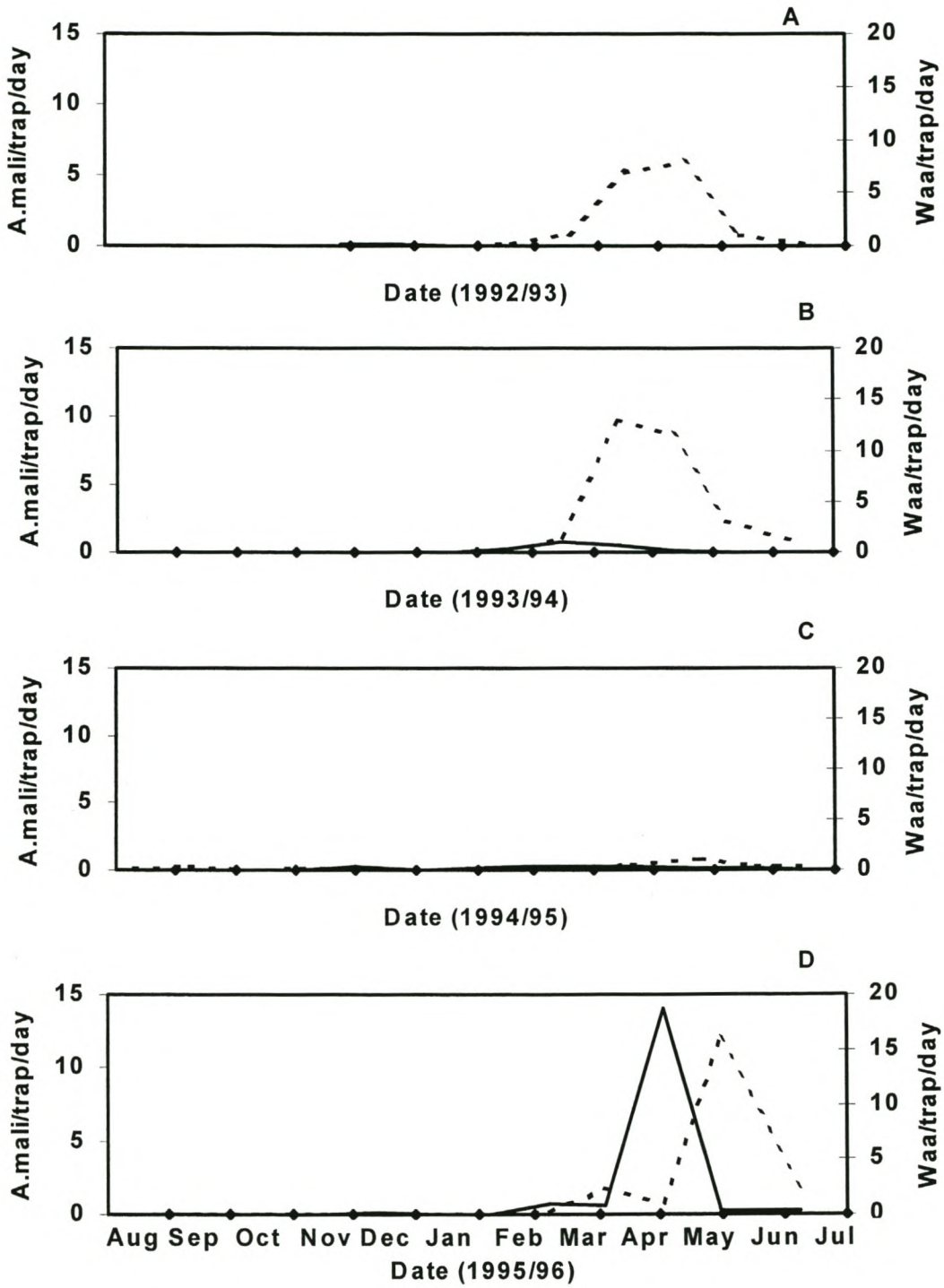

Fig. 4.4.2. Average number of *Eriosoma lanigerum* crawlers, Waa, (solid line) and *Aphelinus mali* adults (broken line) on the yellow sticky traps on Molteno during the 1992/93 (A), 1993/94 (B), 1994/95 (C) and 1995/96 (D) seasons.

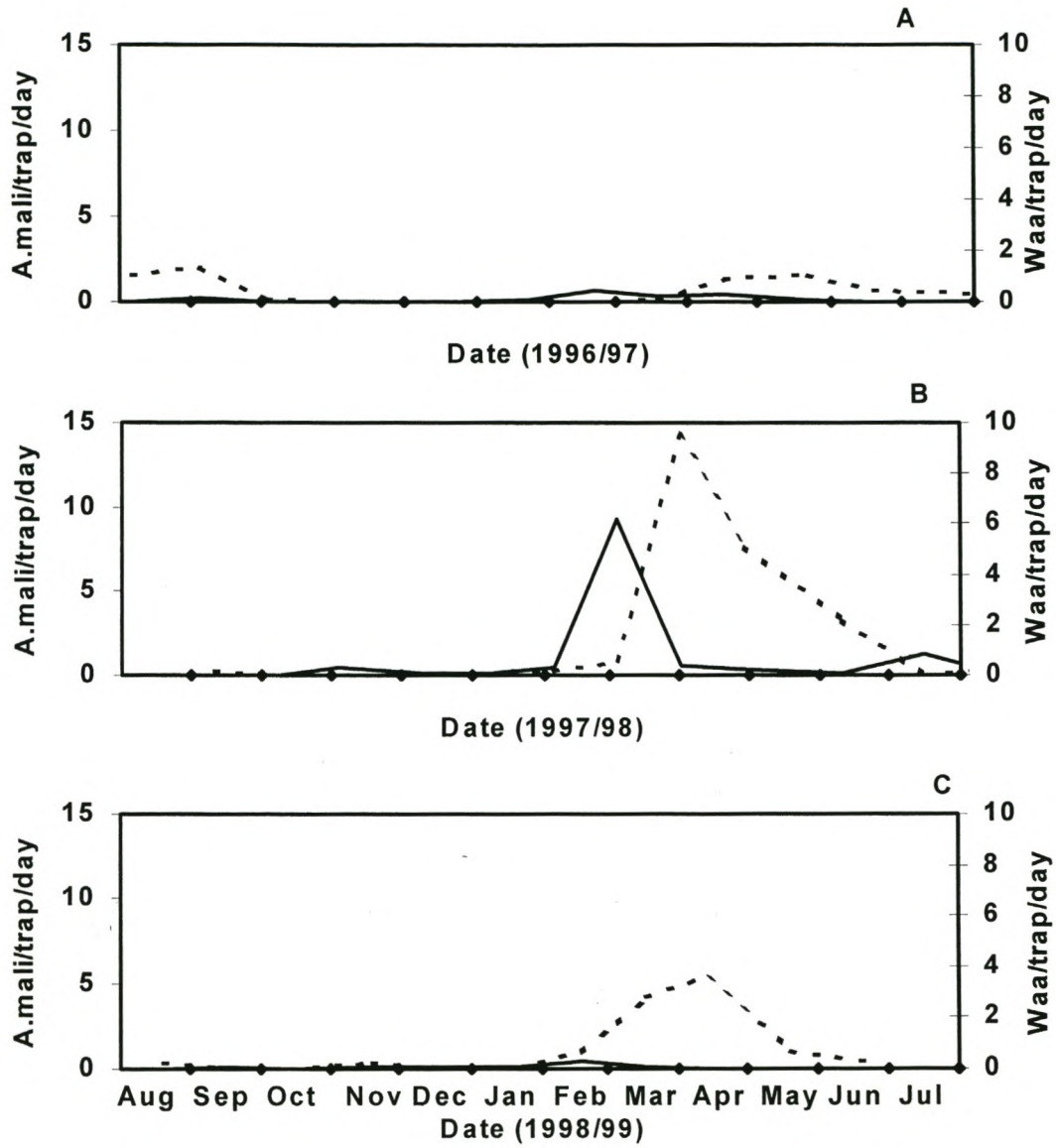

Fig. 4.4.3. Average number of *Eriosoma lanigerum* crawlers, Waa, (solid line) and adult *Aphelinus mali* (broken line) on the yellow sticky traps on Molteno during the 1996/97 (A), 1997/98 (B) and 1998/99 (C) seasons.

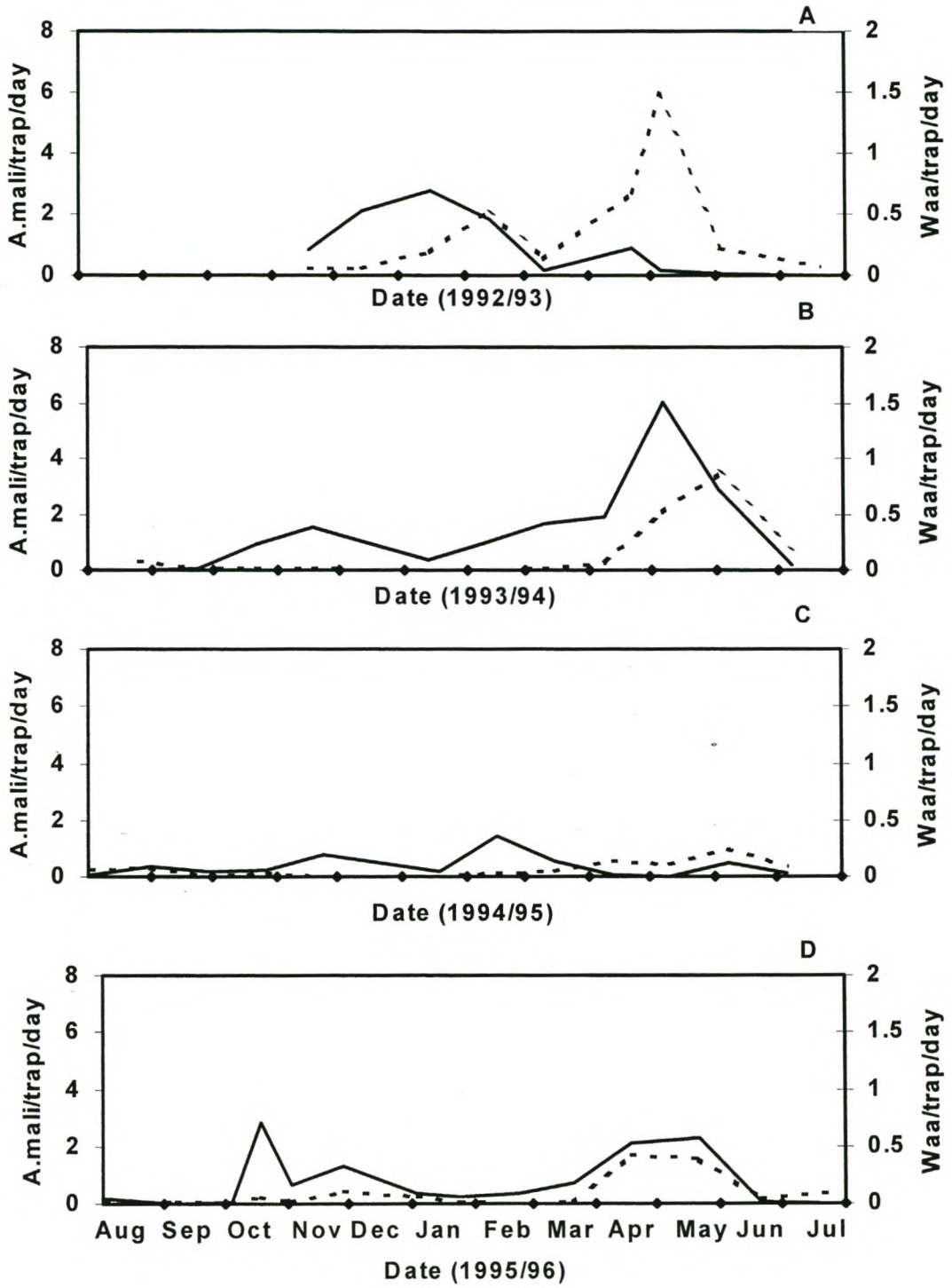

Fig. 4.4.4. Average number of *Eriosoma lanigerum* crawlers, Waa, (solid line) and *Aphelinus mali* adults (broken line) on the yellow sticky traps on Oak Valley during the 1992/93 (A), 1993/94 (B), 1994/95 (C) and 1995/96 (D) seasons.

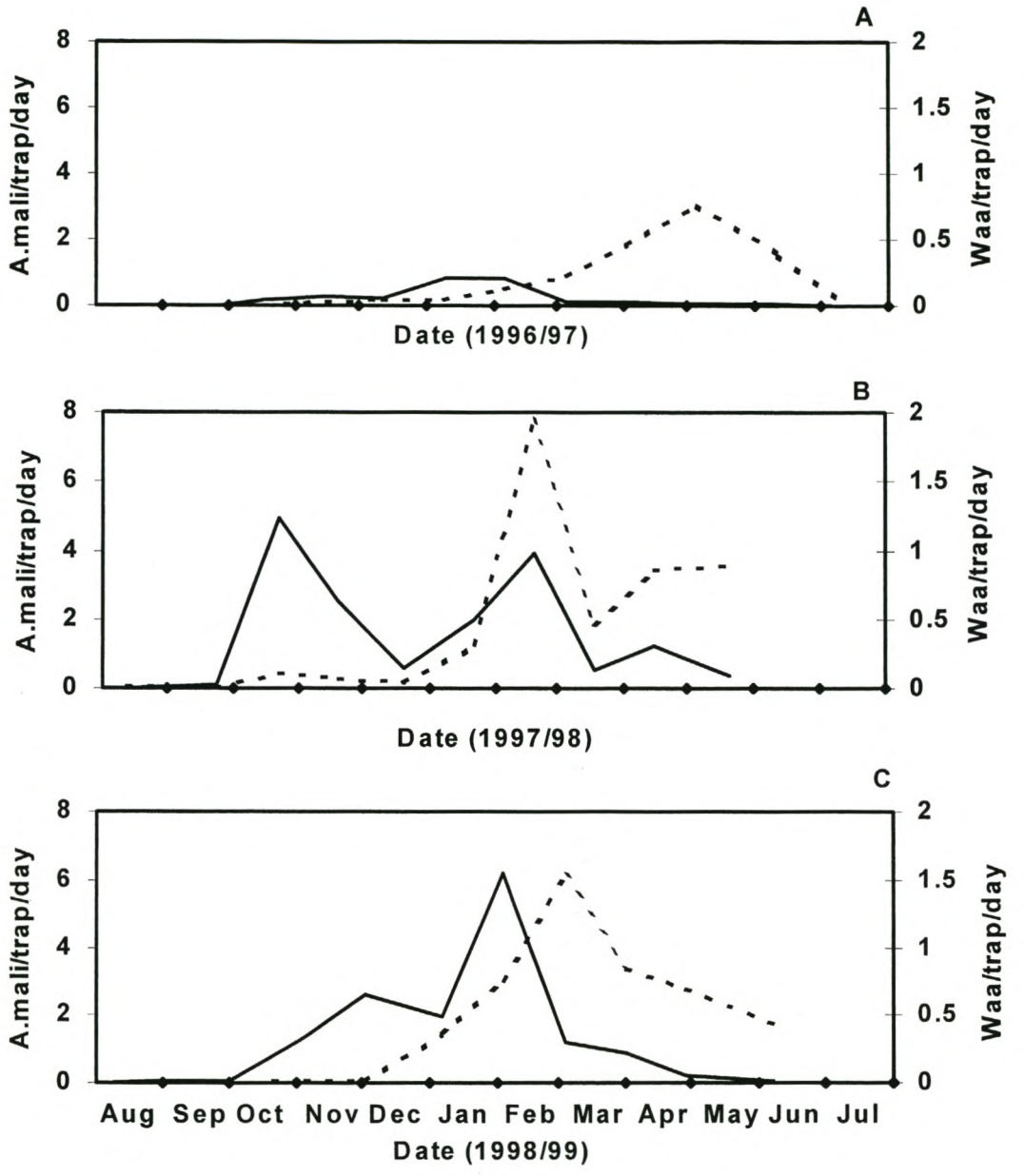

Fig. 4.4.5. Average number of *Eriosoma lanigerum* crawlers, Waa, (solid line) and adult *Aphelinus mali* (broken line) on the yellow sticky traps on Oak Valley during the 1996/97 (A), 1997/98 (B) and 1998/99 (C) seasons.

Very low numbers of parasitoids were recorded during the 1994/95 (Fig. 4.4.2C) and 1996/97 (Fig. 4.4.3A) seasons, while the highest number was recorded during April of the 1997/98 season (Fig. 4.4.3B).

On Oak Valley the number of *A mali* recorded in autumn was always higher than the number of *E. lanigerum* crawlers (Fig. 4.4.4 and 4.4.5). *A. mali* numbers usually increased from the end of summer and reached a peak soon afterwards. They appeared later during the 1993/94, 1995/96 and 1996/97 seasons (Fig. 4.4.4B, D and 4.4.5A) than during other seasons. Chemical sprays applied for the control of *E. lanigerum* (Table 4.3) caused a decline in the number of parasitoids recorded on the yellow traps (Fig. 4.4.4 A, C, D and 4.4.5 B, D).

4.4 Discussion

There was a general phenological pattern of *E. lanigerum* abundance in the Elgin area. In spring large numbers of *E. lanigerum* crawlers moved up into the apple trees from the roots, as was found by Greenslade (1936), Lal & Singh (1945) and Bhardwaj *et al.* (1995). Peak crawler numbers moving up the trunk were usually recorded between the end of October and the end of November. A second and sometimes even a third peak of crawlers were recorded moving up from the roots at the end of summer and during autumn (Figs. 4.1.3A, 4.1.4B and 4.1.5B) in some seasons. However, the third peak coincided with the time colony numbers were high in the aerial parts of the trees. Therefore it was possible that crawlers dispersing from these colonies and/or crawlers falling from large colonies in the trees migrated up the trunks of the trees. Schoene & Underhill (1935) have shown that crowded conditions and the waxy filaments render the crawlers helpless so that they lose their foothold and drop to the ground. Hoyt & Madsen (1960) reported two peak times for upward

migration, one during early summer and a second in mid-summer. A general upward movement again during late summer and autumn was also recorded in Richmond, State of Virginia, U.S.A. (Schoene & Underhill 1935) and India (Bhardwaj *et al.* 1995).

The downward movement of crawlers, measured by the top sticky strips, was not pronounced and may have been due to random movement of crawlers as it occurred at any time when colonies were numerous in the trees. In Virginia a pronounced downward movement usually followed a general heavy infestation in the aerial parts of the trees (Schoene & Underhill 1935). The downward movement may also be reduced as a result of the parasitism by *A. mali* which reduced the number of reproducing *E. lanigerum* in the trees (Hoyt & Madsen 1960).

Colonies of *E. lanigerum* in the trees were found in small numbers during December but they only started to increase from mid- to end-summer. Parasitised colonies were mostly recorded from the end of February of each season. Peak *E. lanigerum* populations occurred from the end of March until April. This was also the time when most of the colonies were parasitised and when the highest numbers of adult parasitoids were recorded on the yellow traps.

After April colony numbers declined when cooler weather set in. The number of progeny, reproductive rate and developmental time of *E. lanigerum* are all influenced by temperature (Chapter 3, Baker 1915, Marcovitch 1934, Bodenheimer 1947, Evenhuis 1958, Gautam & Verma 1983). Parasitism by *A. mali* can also reduce colonies by removing large numbers of reproducing female *E. lanigerum* (Hoyt & Madsen 1960). The appearance of large numbers of winged *E. lanigerum* females in autumn also contributed to the reduction in colonies (Hely *et al.* 1982, Thwaite & Bower 1983, Asante 1994) as these individuals do not produce crawlers. The

physiological state of the apple tree (low quality food in the form of amino acids) at this time of the season could also reduce the rate of reproduction of *E. lanigerum* (Evenhuis 1962, Asante 1994). In Australia the populations declined at the end of autumn through winter, during which time the apple trees shed their leaves and become dormant (Asante 1994).

After the initial decline in *E. lanigerum* colony numbers they may increase again slightly at the end of autumn. *A. mali* becomes less active at temperatures below 25°C while reproduction of E. *lanigerum* is still high (Chapter 3, Bodenheimer 1947, Bonnemaison 1965, Walker *et al.* 1988, Asante *et al.* 1991). Many of the parasitoids also entered diapause (Chapter 8, Evenhuis 1962) and many aphids could escape parasitism.

The *E. lanigerum* population, as well as the number of adult parasitoids, stayed low throughout the winter until the end of August or early September. During this period overwintering *E. lanigerum* colonies were also reduced when the apple trees were pruned (Greenslade 1936) and chemicals were applied to combat delayed foliation, *Q. perniciosus* and *Pseudococcus* pests. Therefore, there was low survival from the aerial population of the previous season. The initial infestations of the aerial parts during early summer were from the first instar crawlers migrating up into the trees during spring or early summer.

The *E. lanigerum* crawlers recorded on the cylindrical sticky traps and yellow sticky traps provided strong evidence for wind dispersal. *E. lanigerum* crawlers were recorded during spring when crawlers moved up from the roots into the trees. This supported observations by Georgala (1953) and Nel (1983). These wind blown crawlers can give rise to new infestations in the aerial parts of trees as well as on the roots if they land on the soil surface and enter the ground through cracks.

During early spring, when *A. mali* appeared after the termination of diapause as recorded on the yellow traps (Fig. 4.4.3A) numbers of *E. lanigerum* were low and the parasitoids could probably not find sufficient hosts so that their numbers remained low. This was also found in China (Lung *et al.* 1960) and in the Netherlands (Evenhuis 1962). In addition, early season sprays against other pests, such as codling moth, could further have reduced the parasitoid numbers (Bengston 1960, Lower 1968, Hely *et al.* 1982, Chapter 10). As a result, the parasitoid could not prevent *E. lanigerum* from reaching high numbers after large numbers of crawlers moved up into the trees.

Biological control is most likely to be successful when the *E. lanigerum* population is distributed in numerous small colonies and few large ones (Mueller *et al.* 1992). This implies that biological control must begin early in the season when the average size of *E. lanigerum* colonies is small, and also that parasitism must persist unabated to restrict colony growth (Mueller *et al.* 1992).

The general phenological pattern was influenced by natural factors like rain and temperature, as well as the application of fruit weevil barriers and chemical sprays. Rainfall during spring and early summer appeared to affect crawler movement into the trees. This was also found in India (Bhardwaj *et al.* 1995). The late start of upward migration during the 1996/97 season (end December) on Molteno (Fig. 4.1.3B) followed a period of high rainfall from September until December 1996 (Table 4.1). The peak number of crawlers moving up the trees during spring of each season on Molteno in relation to the average rainfall during September, October and November of the corresponding seasons (Fig. 4.5.1) showed no clear pattern. However, on Oak Valley there appeared to be a non-linear inverse relationship between rainfall during September, October and November and the maximum number

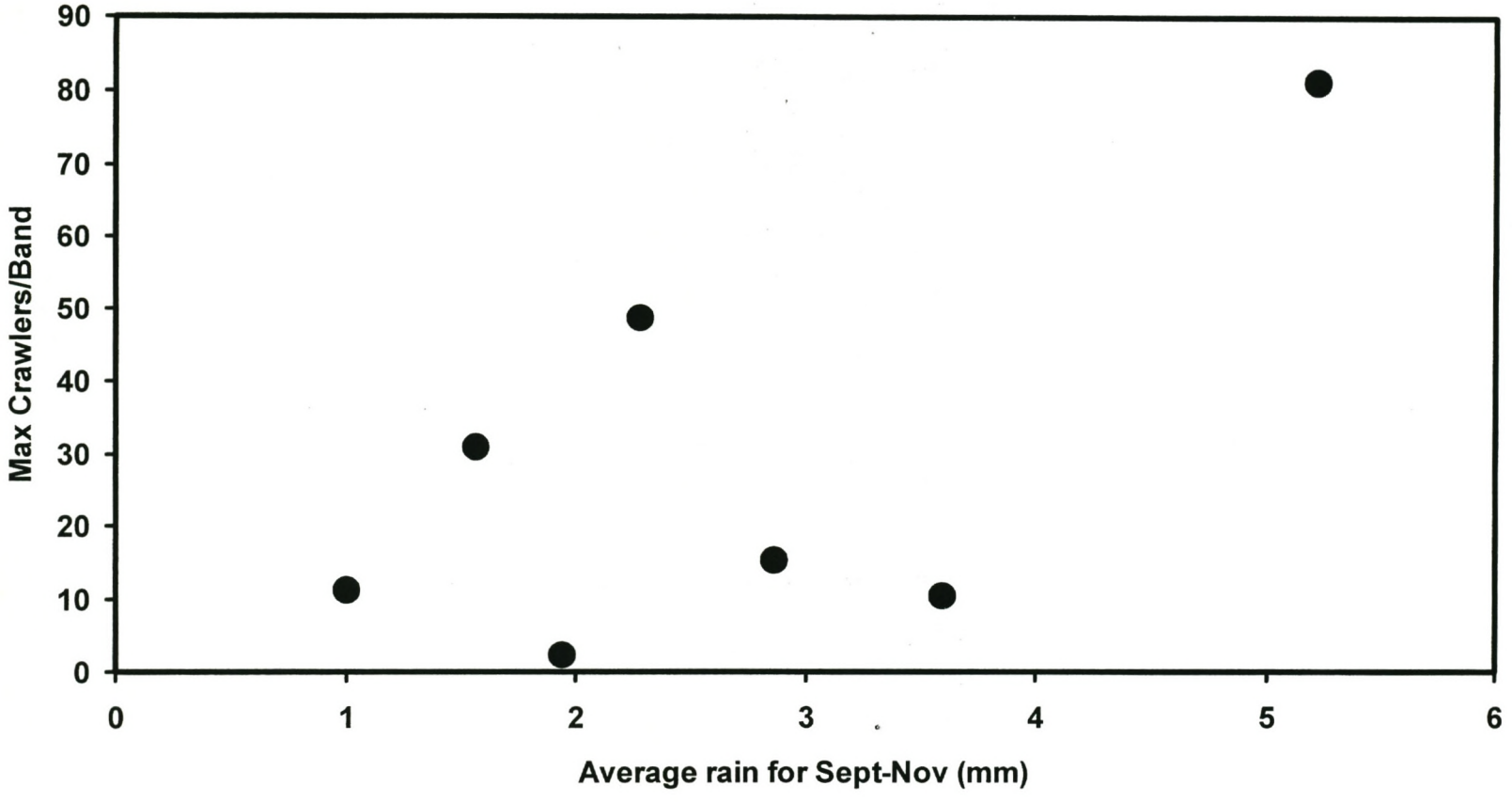

Fig. 4.5.1. Relationship between maximum number of *Eriosoma lanigerum* crawlers moving up the apple trees and the average rainfall for the months September to November each season on Molteno.

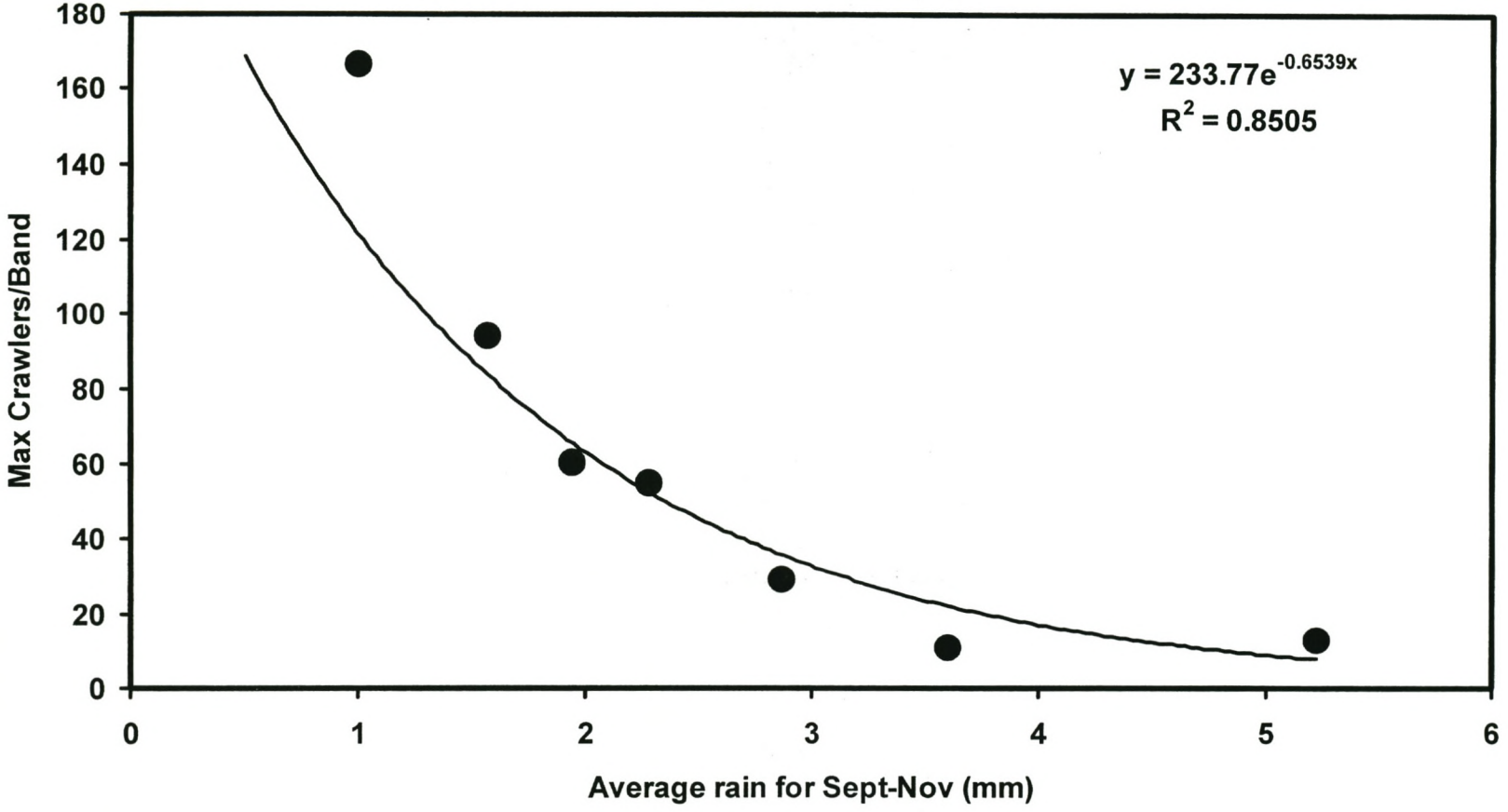

Fig. 4.5.2. The correlation between the maximum number of *Eriosoma lanigerum* crawlers moving up the trees and the average rainfall for the months September to November for each season on Oak Valley.

of crawlers moving up the trees during spring (Fig. 4.5.2). Fewer aphids moved up in seasons when rainfall was high during spring than when the spring rainfall was low. Rain at the time of the upward migration has been reported to wash the aphids from the trunk of the apple trees and seal cracks in the soil through which the aphids emerge (Hoyt & Madsen 1960, Bhardwaj *et al.* 1995). The type of soil could also explain differences in the degree of crawler migration on these two farms. Sandy soils inhibit infestations and heavy soils, which crack, favour infestation (Marcovitch 1934). On Oak Valley the clay content of the soil was higher than on Molteno where the ground contained much more gravel (Fig. 4.1). Therefore, the soil on Oak Valley remained damp much longer, which could reduce the underground *E. lanigerum* populations by making the soil more favourable for the development of biological control agents like nematodes and fungi. In addition, when this type of soil stays damp it does not crack and the crawlers cannot leave underground colonies to move up into the trees (Marcovitch 1934, Hoyt & Madsen 1960). The trees on Oak Valley were in most cases larger than those on Molteno and also had a larger canopy. Therefore the area under the trees stayed damp for longer, inhibiting the upward movement of the crawlers for more extended periods of time.

The fruit weevil barriers applied to the trees on Oak Valley during the 1993/94 season influenced the number of crawlers that entered the aerial parts of the trees. This could be seen on the yellow traps which gave an indication of the number of crawlers on the trees. More aphids were recorded on the masking tape bands moving up the trunk during the 1993/94 season (Fig. 4.1.4B) than during the 1992/93 season (Fig. 4.1.4A). However, fewer crawlers were recorded on the yellow traps during the 1993/94 season than during the 1992/93 season (Fig. 4.4.4A and B), indicating that the fruit weevil barriers had a negative effect on the number of aphids that eventually

entered the aerial parts of the apple trees. The fruit weevil barriers cannot prevent all the crawlers from entering the trees (see also Chapter 7) as the large numbers that moved up during the 1993/94 season (Fig. 4.1.4B) covered the bands to such an extent that many crawlers could walk over them. During the 1997/98 season the weevil bands were only painted with the Plantex® at the end of October, which was after most of the crawlers had already moved up (Fig. 4.1.5B). During the 1998/99 season the Plantex® was applied very early in the season (10 June 1998), and ground and debris covered the strips before the upward migration started in spring (Fig. 4.1.5C). As a result, a large number of aphids was still able to enter the aerial parts of the trees to start new colonies (Fig. 4.4.4C). Wind dispersal can also negate the effect of bands.

On Molteno there was no clear correlation between the number of aphids moving up the trunks and the number of colonies in the aerial parts of the trees. However, on Molteno many factors appeared to influence colony numbers in the trees. During the 1992/93 season low numbers of crawlers moved up (Fig. 4.1.2B) and low numbers of colonies were recorded in the trees (Fig. 4.2.2B), while during the 1993/94 season higher numbers of crawlers were recorded (Fig. 4.1.2C) and more colonies were also found than during the previous season (Fig. 4.2.2C). Colony numbers may have been reduced by the chlorpyrifos spray (Table 4.3) applied at the end of October 1993, as this spray may have removed many of the crawlers which had already moved up into the trees. During the 1994/95 season even more crawlers moved up (Fig. 4.1.2D) but the colony numbers recorded in the trees were not as high (Fig. 4.2.2D) as during the previous season (Fig. 4.2.2C). However, the high temperatures recorded during February 1995 (Table 4.2) may have been detrimental to *E. lanigerum* while favouring the parasitoid, *A. mali* (Chap 3 and 8, Marcovitch

1934, Walker *et al.* 1988, Bo & Rongping 1989, Asante *et al.* 1991). This was also observed during the 1997/98 season (Fig. 4.2.3C) when the high temperatures recorded during February (average=22.51°C) were not followed by the sharp increase in colony numbers usually observed during this time of the season. During the 1995/96 season more crawlers (Fig. 4.1.3A) were recorded than in the 1993/94 season (Fig. 4.1.2C) and much higher colony numbers were also recorded (Fig. 4.2.3A). Colony numbers were higher as no chemical sprays (which could have adversely influenced crawler survival in the trees) were applied during this season, as was the case during October 1993 (Table 4.3).

When crawlers moved up the trees late in the season (Fig. 4.1.3B) after temperatures had increased, colony formation appeared to be lower (Fig. 4.2.3B) than when they moved up during spring (Fig. 4.1.3C and 4.2.3C) when temperatures were lower. Blommers (1994) found that the survival of crawlers was low when summer temperatures were high.

On Oak Valley there was also no apparent relationship between the numbers of crawlers moving up into the trees from the roots (Figs. 4.1.4 and 4.1.5) and the number of aerial *E. lanigerum* colonies in the trees (Fig. 4.2.4 and 4.2.5). This may have been due to the chemical sprays applied for *E. lanigerum* control (Table 4.3) which adversely influenced the survival and development of the aphids. However, there were exceptions. During the 1993/94 season high numbers of crawlers were recorded moving up into the trees and very high numbers of colonies were also recorded (Fig. 4.2.4B). In contrast with this low numbers of crawlers were recorded in the 1996/97 season and correspondingly low colony numbers were also recorded (Fig. 4.2.5A). These were the only two seasons during which chemicals were sprayed at a time when they did not affect colony numbers in the trees as they were sprayed too

early (Table 4.3). The sticky fruit weevil barriers applied to the trees during the 1993/94 (Fig. 4.1.5B) season may also have influenced the number of crawlers that eventually reached the aerial parts of the tree (see above).

The phenological pattern of occurrence of *E. lanigerum* and *A. mali* is summarised in Fig. 4.6.1. Crawler movement from the roots into the trees occurred from October to June with peak movement from October to December. The peak movement up from the roots also coincided with a peak in aerial movement of crawlers recorded on the yellow sticky traps. There was a second and longer peak in aerial movement of crawlers from January to mid-May. This coincided with peak occurrence of colonies in the trees. Peak numbers of unparasitised colonies were present from December to February, while high numbers of parasitised colonies occurred from February until mid-May when the trees started entering dormancy. *A. mali* was recorded on the yellow sticky traps from January until August, while peak activity was from February until June. Even low levels of activity of *A. mali* during December was later than the initial peak appearance of colonies during December, which largely explained the limited level of biological control of *E. lanigerum* by *A. mali*.

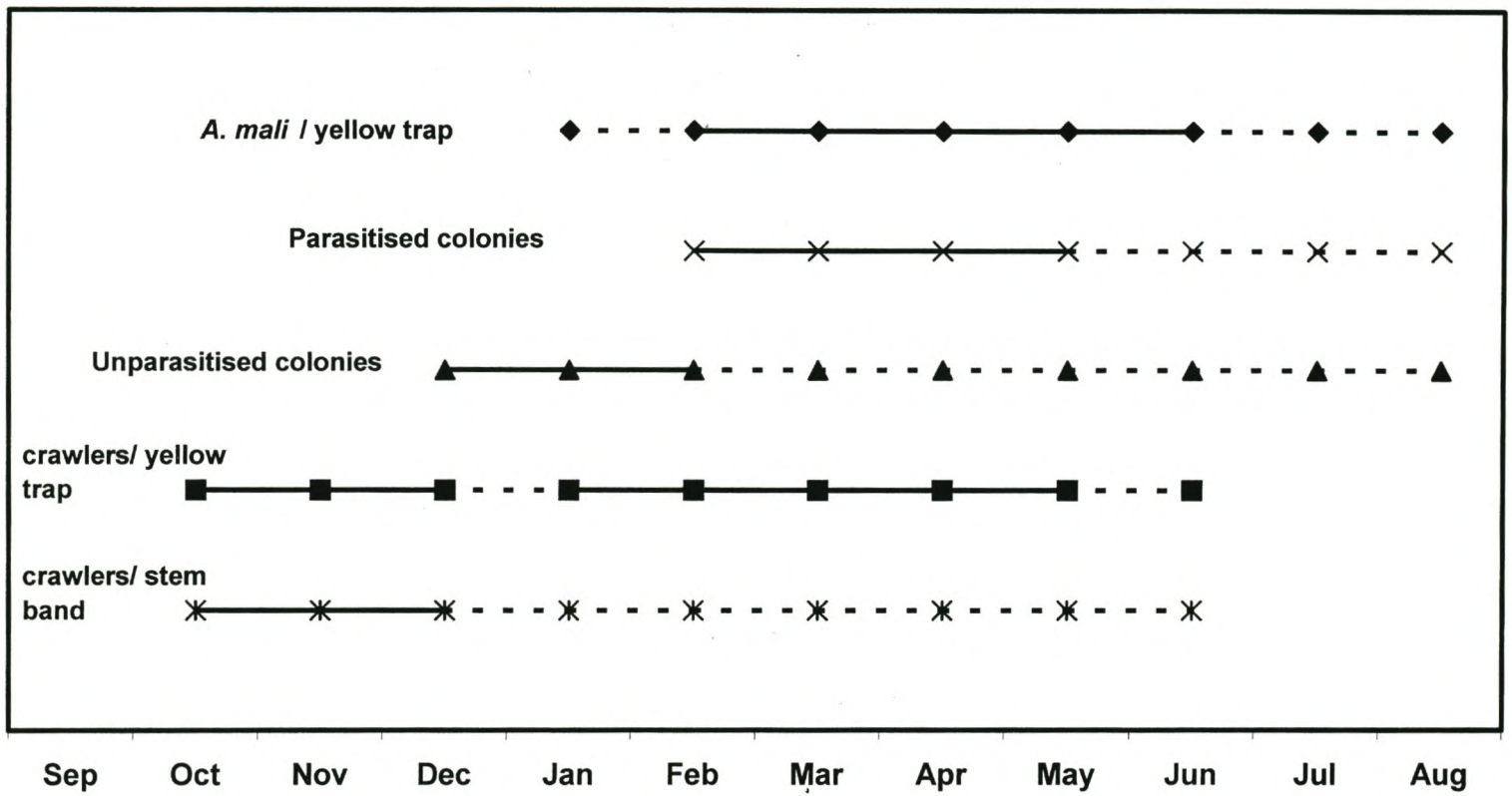

Fig. 4.6.1. Periods during which *Eriosoma lanigerum* crawlers moved up from the roots to the aerial parts of the trees (crawlers/ stem band); there was aerial movement of crawlers (crawlers/yellow trap); unparasitised and parasitised colonies were recorded; *Aphelinus mali* was active (*A. mali* / yellow trap). Broken line = present; solid line = peak numbers.

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CHAPTER 5

THE DEVELOPMENT OF A SAMPLING SYSTEM FOR MONITORING POPULATION LEVELS OF THE WOOLLY APPLE APHID, *ERIOSOMA LANIGERUM* (HAUSMANN).

5.1 Introduction

Woolly apple aphid, *Eriosoma lanigerum* (Hausmann) feeds underground in colonies on the roots of apple trees. During spring and throughout the summer crawlers (first instar nymphs) move up the trunks of the trees and settle in leaf axils, pruning wounds and injured bark where they complete their development. They remain there as adults and their parthenogenic reproduction gives rise to colonies where the original crawlers settled. These aphids produce white, waxy filaments, making the colonies in which they live easily visible.

The feeding of *E. lanigerum* in the leaf axils destroys the buds, adversely affecting the following season's crop. Where heavy infestations are encountered, severe crop losses can occur. The colonies are attacked by a parasitic wasp, *Aphelinus mali* (Haldeman). However, *A. mali* often appears too late in the season to prevent colony formation and

bud damage, and corrective sprays are often required. The most commonly used chemicals are endosulfan and, until recently, chlorpyrifos. Both are contact chemicals with short residual action, especially the former. Correct timing of the sprays is therefore important because colonies do not appear at the same time in different orchards and during different seasons (Chapter 4). This can only be determined by regular monitoring of population levels. The object of the present study was therefore to develop a sampling system with known levels of error for monitoring *E. lanigerum* populations in commercial apple orchards, so that producers can determine whether or not spray applications are required.

5.2 Material and methods

The study was conducted over a three year period (1991/1992 until 1993/94). Blocks of commercial apple orchards on four different farms were used. The blocks were divided into sub-sections not exceeding two hectares. Twenty-five evenly spaced trees were selected as sample trees in each of the two-hectare sections. This sampling pattern was decided upon as it is used for monitoring population levels of other pests (Pringle & Giliomee 1992). A total of fifteen such two-hectare units were used throughout the study. Granny Smith trees were used in all except one unit, where Starking trees were sampled.

Sampling was conducted by counting colonies on half of each tree. In Australia a significant relationship was found between the total number of *E. lanigerum* and the number of colonies in the trees (Asante *et al.* 1992). Therefore, counts of colonies can

provide an estimate of the aphid population. Colonies in wounds were counted separately from those in leaf axils, as were parasitised and unparasitised colonies. Therefore there were four categories, unparasitised and parasitised colonies in leaf axils and unparasitised and parasitised colonies in wounds. Sampling was conducted at intervals of one to three weeks throughout each of the three seasons, starting in November and ending after the end of March.

The index, *D*, was used as a measure of sampling error (Iwao & Kuno 1971),

$$D = \frac{S\bar{x}}{\bar{x}} \quad (1),$$

where $S\bar{x} = \sqrt{\frac{S^2}{n}}$,

$\bar{x}$ = average number of insects per sampling unit,

S^2 = sample variance and

n = number of sampling units.

However, expression (1) assumes that the variance, S^2, is stable. This is very seldom the case with counts of insects as the variance is usually related to the sample mean by the expression (Taylor 1961),

$$S^2 = a(\bar{x})^b \quad (2).$$

The constants, a and b, can be estimated from the linear regression,

$$log\ S^2 = log\ (a) + b\ log\ \left(\bar{x}\right) \qquad (3).$$

Substituting (2) into (1) an expression for estimating sampling error, D, for any number of trees (n) and a given population level (average colonies per half tree), $\bar{x}$, can be obtained as follows,

$$D = \frac{1}{\bar{x}}\sqrt{\frac{a\left(\bar{x}\right)^b}{n}} \qquad (4).$$

The average number of colonies per half tree and the variances were estimated from the counts of colonies on the 25 trees obtained from each two-hectare sampling unit on each date. The regression given in expression (3) was fitted to these data to obtain estimates of $log\ (a)$ and b. In the regression analysis dummy variables (Gujarati 1970a, b; Neter & Wasserman 1974) were used to determine whether or not data pertaining to colonies in axils would produce different regression coefficients ($log\ (a)$ and b) from those in wounds, or whether or not parasitism affected the regression coefficients. Differences in these coefficients would produce differences in the sampling error, D, estimated from (4). The sampling error, D, was then estimated for a range of values of n (number of trees sampled) to examine the effects of changing the number of trees on which colonies are counted on sampling error. A value of $\bar{x} = 5$ unparasitised colonies

per half tree was used in expression (4) as the economic threshold (ET). In some cases when this threshold was exceeded, there were very heavily infested trees. However, at averages of below 5 colonies per half tree, heavily infested trees were not seen.

The object of sampling in pest management is usually to enable a decision to be made as to whether or not to intervene, for example by applying chemical sprays. Operating characteristic curves (OC-curves) provide probability levels that the decision not to intervene will be correct for any pest population density estimated from the sample (Binns *et al.* 2000).

OC-curves can be estimated using

$$z = \frac{\bar{x} - ET}{\sqrt{\frac{S^2}{n}}} \quad (5)$$

Equation (2) was substituted into (5) after replacing $\bar{x}$ in (2) with the ET to produce,

$$z = \frac{\bar{x} - ET}{\sqrt{\frac{a(ET)^b}{n}}} \quad (6).$$

Estimates of z were then obtained for a range of values of $\bar{x}$ from (6) (Binns *et al.* 2000) and the probability levels were obtained from one-tailed normal probability tables. In (6)

the value of ET = 5 unparasitised colonies per half tree was used as the economic threshold for the reasons given above, and $n = 25$ trees sampled was used.

A monitoring system based on examining trees for infestation will be quicker than counting colonies. Therefore, the relationship between the average number of colonies per half tree and the proportion of infested trees was investigated. Binns *et al.* (2000) give an expression which provides a link between actual counts and presence-absence data,

$$ln(-ln(1-p)) = A + B\, ln(\bar{x}) \qquad (7)$$

where *ln* is the base of the natural logarithm, p is the proportion of trees infested by unparasitised *E. lanigerum* colonies in the axils per half tree, A and B are regression coefficients and $\bar{x}$ is the average number of unparasitised colonies in the axils per half tree. The linear regression of $ln(\bar{x})$ as the independent variable and $ln\{-ln(1-p)\}$ as the dependant variable was estimated. Binns *et al.* (2000) showed that the proportion of infested trees, p, for any value of $\bar{x}$ can be estimated using,

$$p = 1 - \exp\left[-\left\{\exp(A)(\bar{x})^{B}\right\}\right] \qquad (8)$$

An estimate of the economic threshold of the proportion of infested trees was obtained by substituting the average number of unparasitised *E. lanigerum* colonies $(\bar{x})$ with the economic threshold (ET) of 5 unparasitised colonies per half tree into (8).

The presence-absence sampling plan was also evaluated by using operating characteristic (OC) curves. The general expression for estimating OC curves is given by (5). Expression (8) was used to provide an estimate of a range of proportions of infested trees and the ET was replaced by the estimate of the proportion of infested units at the ET in the numerator. Binns *et al.* (2000) suggested that the expression for the standard error of the mean in the binomial distribution could be used.

$$SE(\bar{x}) = \sqrt{\frac{p(1-p)}{n}} \quad (9).$$

In (9) p was substituted with (8) using the ET as $\bar{x}$. This produced the following expression for z,

$$z = \frac{\left[1-\exp\left\{-\left(\exp(A)(\bar{x})^{B}\right)\right\}\right]-\left[1-\exp\left\{-\left(\exp(A)(ET)^{B}\right)\right\}\right]}{\sqrt{\frac{\left[1-\exp\left\{-\left(\exp(A)(ET)^{B}\right)\right\}\right]\left[\exp\left\{-\exp(A)(ET)^{B}\right\}\right]}{n}}} \quad (10).$$

A range of values for $\bar{x}$ was substituted into (10) to produce a range of values for z. The corresponding probability levels for these values of z were obtained from normal

probability tables. The OC-curve was obtained by plotting the values of $(\bar{x})$ in (10) against the probability levels.

5.3 Results

In the full dummy variable regression of $log(S^2)$ on $log\ (\bar{x})$, data pertaining to unparasitised colonies in leaf axils were used for determining the basic regression coefficients. These and the changes in the basic coefficients produced by parasitised colonies in leaf axils, unparasitised and parasitised colonies in wounds together with their significance levels are given in Table 5.1.

Table 5.1. Regression coefficients (*log* (*a*) and *b*) for unparasitised *Eriosoma lanigerum* colonies in leaf axis and changes in these coefficients for parasitised colonies in leaf axils, unparasitised colonies in wounds and parasitised colonies in wounds together with their significance levels (P).

Description	*log* (*a*)	P	*b*	P
Basic coefficients - unparasitised colonies in leaf axils	0.876	<0.001	1.652	<0.001
Change due to parasitised colonies in leaf axils	0.087	0.0011	0.017	0.6072
Change due to unparasitised colonies in wounds	-0.453	<0.001	-0.391	<0.001
Change due to parasitised colonies in wounds	-0.365	<0.001	-0.374	<0.001

Data pertaining to parasitised colonies in leaf axils significantly increased the intercept, *log* (*a*), but did not change the slope, *b*, of the regression of $log(S^2)$ on $log(\bar{x})$ (Table 5.1). In addition, data pertaining to unparasitised and parasitised colonies in wounds significantly reduced the intercept, *log* (*a*), and slope, *b*, of the regression. However, colonies in wounds are not responsible for economic damage. Therefore, all data pertaining to these colonies were omitted from the data set. The remaining data, pertaining only to colonies in leaf axils, were analysed using dummy variables to examine the effects of parasitism in leaf axils on the regression coefficients.

There was good correlation between *log* (S^2) and $log(\bar{x})$ ($R^2 = 0.996$). Parasitism did not affect the slope, *b*, of the regression (P=0.655), but increased the intercept, *log* (*a*), (P=0.005). Therefore, two regression equations with a common slope and different intercepts were obtained,

$log(S^2) = 0.867 + (1.659)\ log\ (\bar{x})$ for unparasitised colonies in leaf axils and

$log\ (S^2) = 0.962 + (1.659)\ log\ (\bar{x})$ for parasitised colonies in leaf axils.

The regression coefficients in the above equations were used to solve expression (4) for different values of *n* (number of trees sampled) and for $\bar{x} = 5$ unparasitised colonies per half tree. The results are illustrated in Fig. 5.1. These data were based on sampling 25 trees per two hectare unit. The sampling error for 25 trees was just over 40 % and from

the shape of the lines it was clear that increasing the number of trees sampled would not lead to a marked increase in precision. Although the regressions for unparasitised and parasitised colonies were different, this was not reflected by markedly different sampling errors (Fig. 5.1).

The OC-curves for the *E. lanigerum* data are given in Fig. 5.2 using an ET of 5 unparasitised colonies per half tree. This curve is relatively flat suggesting that decisions not to intervene when less than 5 colonies per tree are recorded will be unreliable. For example, if 2.5 colonies per half tree were recorded in the sample, the decision not to intervene would be incorrect in 10% of the cases.

The regression given by (7) indicated that there was a good relationship between the proportion of infested trees and average colonies per tree (R^2=0.75, P<0.001) (Fig. 5.3). This regression was

$$ln\{-ln(1-p)\} = -1.4551 + 0.5056\, ln\,(\bar{x}).$$

From expression (8) the economic threshold of $\bar{x}$ = 5 unparasitised colonies per half tree was the equivalent of 41% infested trees. Therefore, based on a sample size of 25 trees per two hectare unit, the economic threshold was reached when 11 of the 25 trees were infested with unparasitised colonies in the axils.

The OC-curves for presence-absence sampling as well as for the sampling of *E. lanigerum* colonies are given in Fig. 5.2. using an ET of 5 unparasitised colonies per tree or 41% infested trees. The OC-curve for presence-absence sampling was similar to that

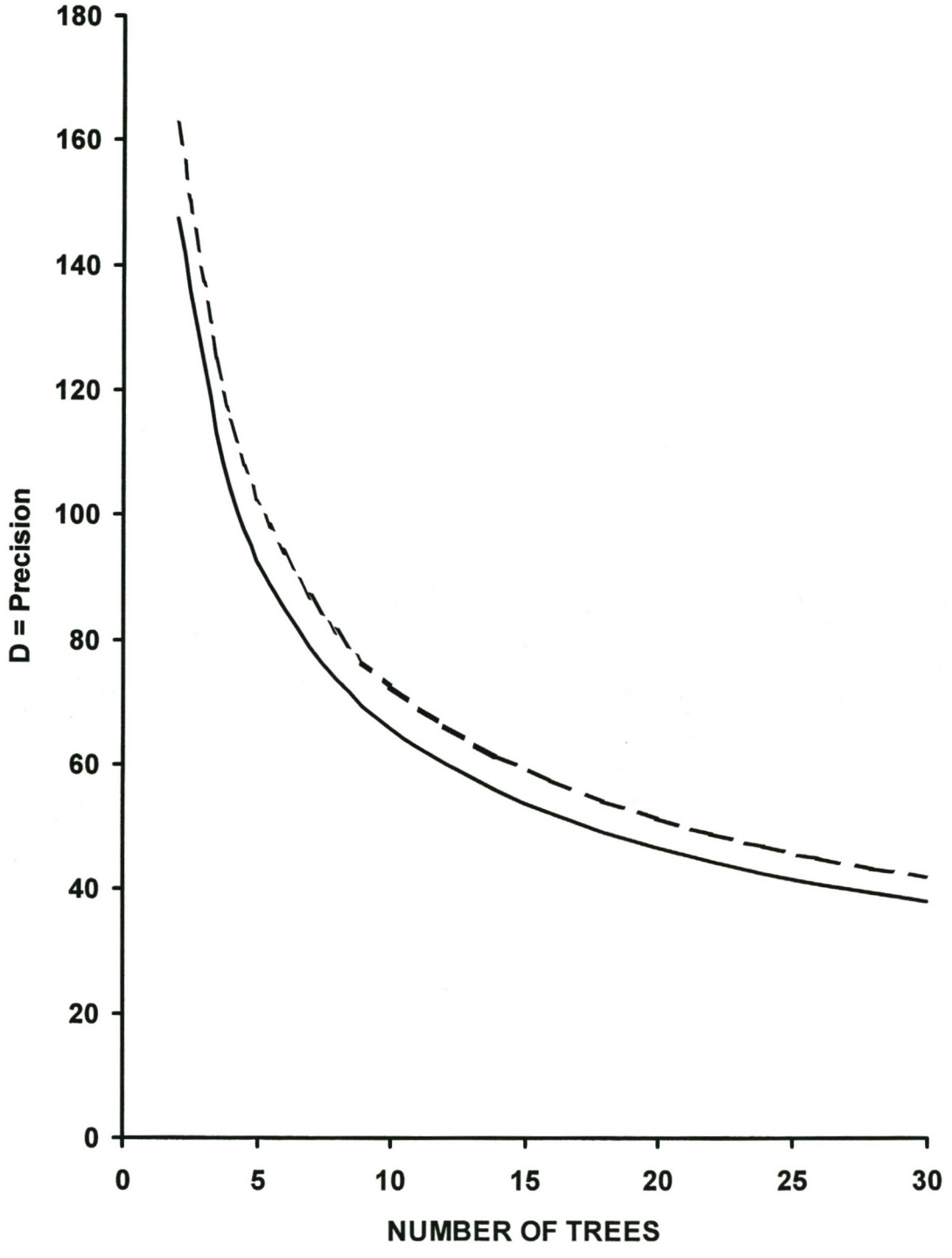

Fig. 5.1. Sampling regression (D) versus number of trees sampled for unparasitised (solid line) and parasitised (dotted line) *Eriosoma lanigerum* colonies in leaf axils.

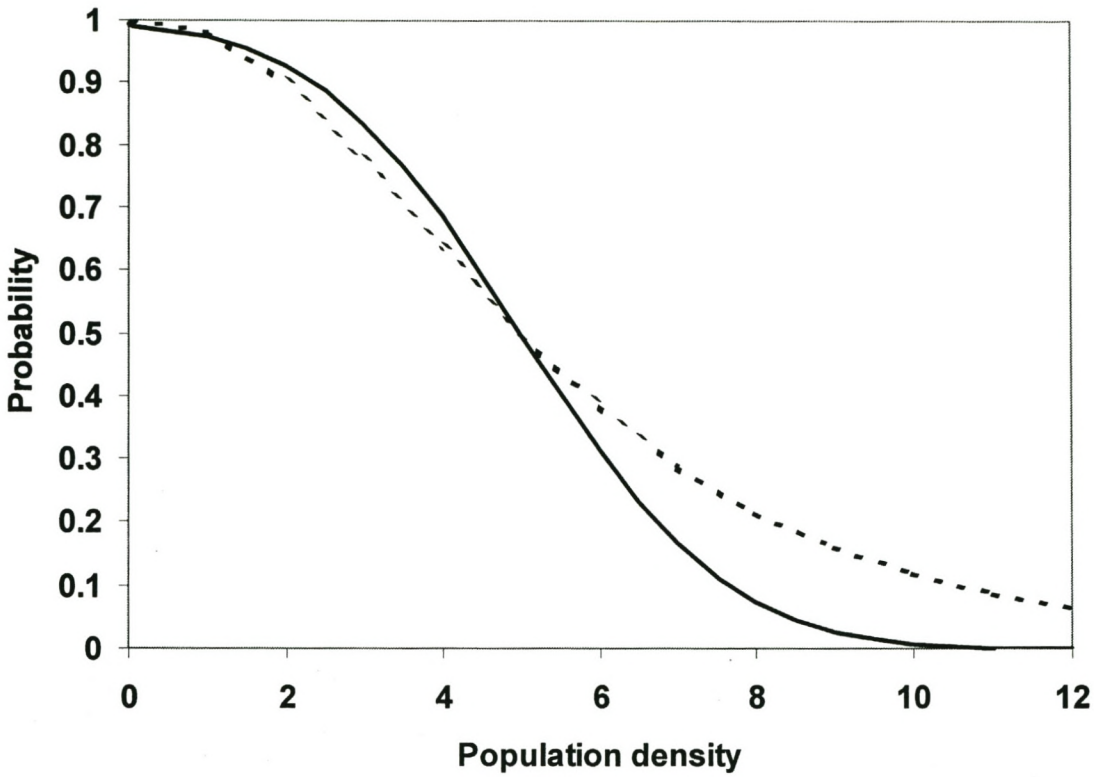

Fig. 5.2. Operating characteristic (OC) curve for sampling of *Eriosoma lanigerum* colonies per half tree (solid line) and presence-absence sampling (broken line) using an economic threshold (ET) of 5 unparasitised colonies per half tree.

produced by the actual counts of colonies in the trees at population levels lower than the ET of 5. This suggests that decisions regarding spraying against *E. lanigerum* will be no less reliable than using counts of colonies.

5.4 Discussion

The sampling error using counts of *E. lanigerum* colonies in leaf axils on one half of 25 trees per 2 ha of apple orchards exceeded the value of 20% (Reusink & Kogan 1994) as acceptable for pest management purposes. This high degree of sampling error is the result of the high intercept value (*log* (*a*)) of 0.876. This usually reflects high back ground variance (Taylor 1984). A feature of *E. lanigerum* infestations is that a tree may have high numbers of colonies, sometimes in excess of 100, while nearby trees are uninfested. Therefore, the high sampling error simply reflects the highly erratic infestation pattern. However, *E. lanigerum* is not a direct pest in that it does not directly affect the marketable product. Although buds are destroyed which would produce part of the following season's crop, in many cases the injured wood can be pruned off during the winter, allowing for a certain degree of compensation. Therefore, in that instance the high degree of sampling error can be tolerated. The reliability of decisions regarding the necessity for intervention will not be seriously compromised using presence-absence sampling, which will reduce the time required for monitoring considerably. This makes this monitoring system attractive, despite the high level of sampling error.

Therefore, *E. lanigerum* can be monitored by determining the percentage infested trees from 25 evenly spaced trees per 2 hectare block and the OC-curve (Fig. 5.2) can be used to determine the risk involved when deciding whether or not to spray.

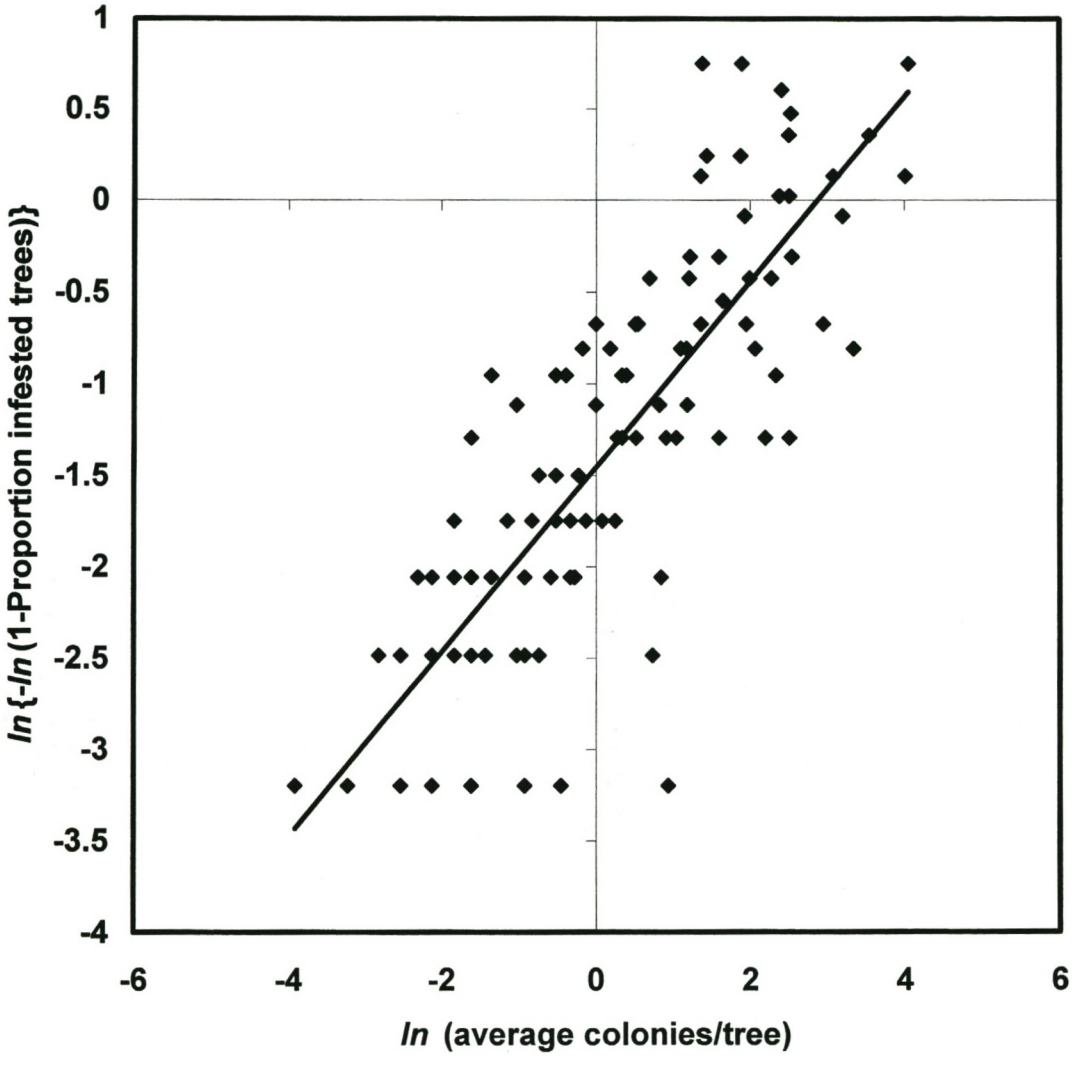

Fig. 5.3. Relationship between *ln* {-*ln* (1-proportion infested trees)} and *ln* (average colonies per tree).

5.5 References

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CHAPTER 6

THE INFLUENCE OF NITROGEN FERTILIZATION, TIME OF YEAR AND ORIGIN OF CRAWLERS ON THE COLONISATION OF THE ABOVE GROUND PARTS OF APPLE TREES BY *ERIOSOMA LANIGERUM* (HAUSMANN).

6.1. Introduction

Nitrogen is one of the main nutrients in plant tissue essential for phytophagous insects (Strong *et al.* 1984), and the development of aphids has often been shown to be closely correlated with the nitrogen content of the host plants.

In general, aphid colonies on woody plants show seasonal changes, with high population levels occurring together with high nitrogen levels during spring and autumn, and low levels during summer when nitrogen levels are low (Lindeman 1948, Mittler 1958).

Many researchers have found that colonies of *Eriosoma lanigerum* (Hausmann) were numerous during spring but that numbers dropped during summer. Numbers increased again during autumn until colder winter weather set in (Lohrenz

1911, Marchal 1929, Marcovitch 1934, Lal & Singh 1946, Bodenheimer 1947, Evenhuis 1958 and 1962, El-Haidari *et al.* 1978, Mueller *et al.* 1992, Brown & Schmitt 1994). The reasons for this low activity during summer are not clear. According to Marchal (1929) and Walker *et al.* (1988) heat caused a reduction in the rate of reproduction, but according to others it is the unfavourable physiological conditions of the host plant (Evenhuis 1958, 1962) as well as high temperatures and low humidity (Marchal 1929, El-Haidari *et al.* 1978) that were detrimental to population development. Lal and Singh (1946) found that the reduction in colony numbers was caused by high humidity. Lohrenz (1911) observed that *E. lanigerum* as well as other apple aphids that were numerous disappeared when the weather became warm and dry.

Asante (1994) found that fecundity and population growth was highest during spring, and that during March aphids showed a dramatic increase in size. This might have been the result of the availability of high quality food (amino acid content) in the host plant during those times of the year. Miles (1972) showed that *E. lanigerum* galls had higher concentrations of amino acids than ungalled portions of the apple trees and that these galls were more readily colonised by aphids. Galls are beneficial to *E. lanigerum* because of the increased nutritional value and greater susceptibility to infestation caused by higher concentrations of nitrogen. Rinallo *et al.* (1995) found that apple trees with higher nitrogen levels and relatively low levels of phenolics were more likely to be attacked by *E. lanigerum.* Sen Gupta and Miles (1975) also reported that *E. lanigerum* had a preference for cultivars with higher amounts of soluble nitrogenous compounds.

In the Western Cape chemical products high in nitrogen, such as calcium nitrate, are applied regularly from early to late summer for the control of bitterpit, a

disease caused by calcium deficiency. During this time large numbers of *E. lanigerum* crawlers move up from the roots into the trees (Chapter 4) to start new colonies. It is possible that materials containing nitrogen, when applied to apple trees, may influence the colonisation of apple trees by *E. lanigerum*. Therefore, the influence of calcium nitrate used for bitter pit control on the initial colonisation of *E. lanigerum* crawlers originating from roots and from twigs was investigated.

6.2. Material and methods

Small apple trees, removed from the ground at the end of winter, were kept in bark chips in a cool room (1-4°C) until needed. During the first and third seasons of the experiment Granny Smith apple seedlings were used and Royal Gala were used during the second season. In the first and second seasons the trees were 1 to 1.5 m high and were cut to about 15 cm when planted. During the third season the trees were about 60 cm high and were also cut to about 15 cm. Forty of these trees were planted in plastic pots (15-29 cm diameter) five weeks before an experiment was started. The pots were placed on metal trays in an air-conditioned room with a temperature between 21 and 23°C. The trees were watered twice weekly. Two weeks, and one week prior to the beginning of the experiment and on the day the experiment was started, 20 trees were sprayed until runoff with a liquid formulation of calcium nitrate, Calnitro®, at a rate of 675ml/l water. The formulation contained 175 g/l calcium and 124 g/l nitrogen. Small, black paper cones (1.5-2.0 cm in diameter) were placed around the stems of each of the trees. Ten sprayed trees and ten unsprayed trees were infested with 10 first instar crawlers from apple tree roots collected in a commercial

orchard by placing the crawlers individually in the axils of the leaves. The other 20 trees were infested in the same way with crawlers removed from apple twigs from an apple orchard when aerial colonies were available. If crawlers dropped off while being placed, or moved down the stem after being placed, they encountered the paper cone and had the opportunity to move back up and settle in a leaf axil. After one week the trees were inspected with a microscope and the crawlers that had settled and started feeding were counted. The experiment with crawlers from roots was repeated at monthly intervals from October 1996 until March 1997, from October 1997 until February 1998 and from October 1998 until February 1999.

Crawlers on apple twigs from commercial orchards became available during January 1997, December 1997 and January 1999. It was later discovered that endosulfan had been applied shortly before the aphids were collected from the field during December 1997. These results were ignored as the mortality was exceptionally high. In addition, during February and March 1998, the trees in the laboratory were contaminated with *E. lanigerum* and those results were also ignored. In all cases except during January 1997 the trees were sprayed with Calnitro® prior to infestation. In the January 1997 experiment the trees were sprayed after infestation and it appeared as if some of the aphids were dislodged by the spray. These data were also ignored.

Data for crawlers from the roots were analysed using a factorial analysis with treatment (spray or no spray) and dates as main effects. In experiments in which crawlers from the twigs were available the data were also analysed in a factorial design but with treatment, dates and origin of crawlers (from roots or from twigs) as main effects.

6.3. Results

During the first season survival of crawlers from colonies on roots was highest during October and November 1996 and again during February 1997 for both the treated and untreated trees (Fig. 6.1A). These differences between dates were significant ($F_{4;89}$=9.34, P<0.001). Survival of crawlers during the second season was significantly higher during October and November 1997 than at other times of the year ($F_{(3;71)}$=8.99, P<0.001) (Fig. 6.1B). During the 1998/99 experiment (third season) a different trend was observed in the survival of the crawlers during the year (Fig. 6.1C). Survival was the lowest in October 1998 and increased until January 1999. During this season very low numbers of crawlers moved up into the trees (Chapter 4) and it was difficult to find crawlers from roots during October 1998. The difference between dates in 1998/99 was also significant ($F_{(4,88)}$ = 3.65, P=0.008).

These results indicated that there were differences in survival between dates. Usually there was higher survival of crawlers collected during spring than of those collected during December. This was not the case when there was low woolly apple aphid activity on the roots during spring, as occurred during the third season. This higher spring survival coincided with the early summer crawler movement from the roots into the trees (Chapter 4). Higher survival during late summer coincided with the second upward movement that was sometimes found in the orchard (Chapter 4, Hoyt & Madsen 1960). Lindeman (1948) and Mittler (1958) also found that colony formation on woody plants showed seasonal changes. The *E. lanigerum* population on the roots of apple trees was higher during spring and summer (Damavandian 2000).

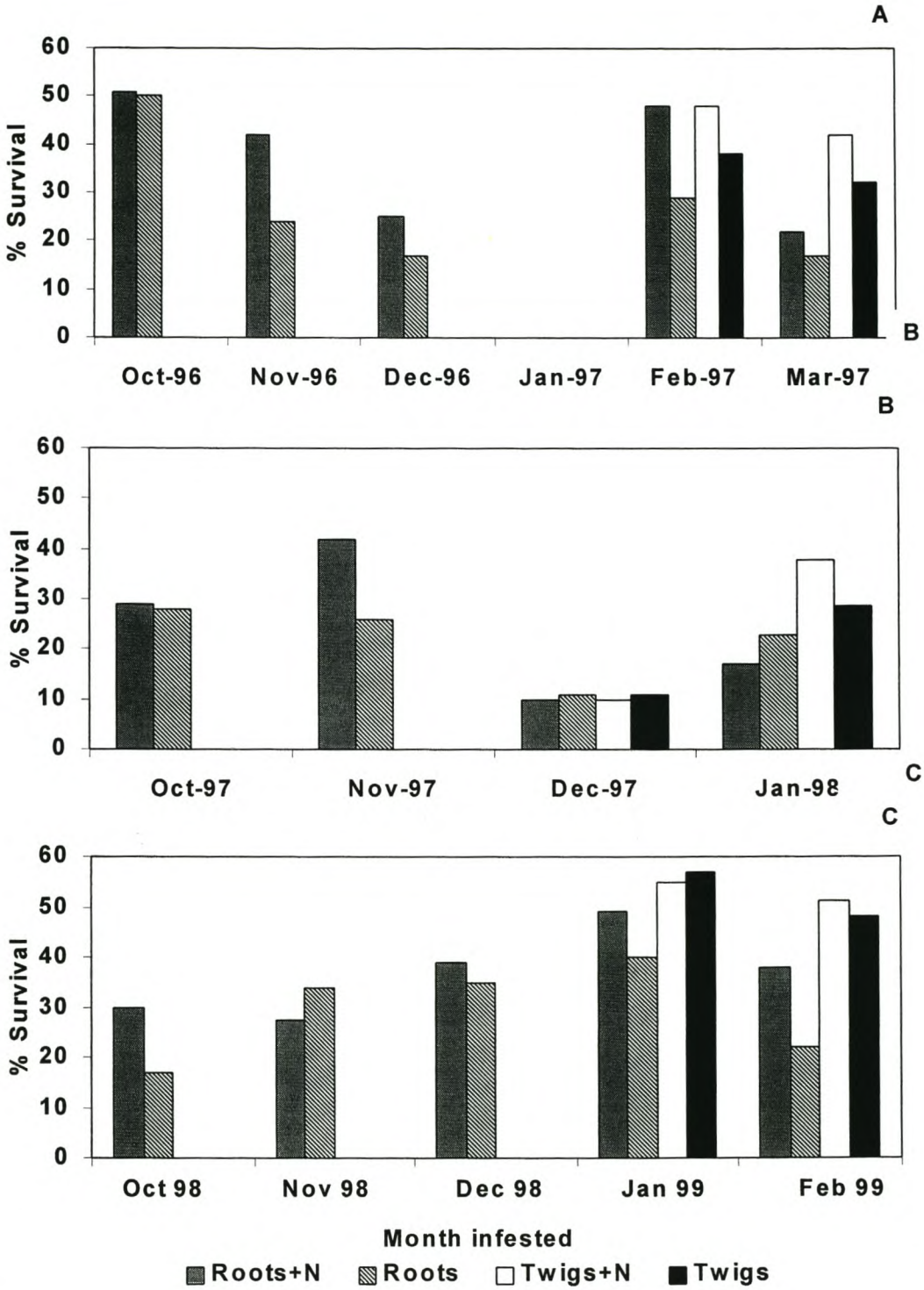

Fig. 6.1. Per cent survival of *Eriosoma lanigerum* crawlers from the roots and twig colonies on apple trees treated with nitrogen (Calnitro®), and those left untreated in the 1996/97 (A), 1997/98 (B) and 1998/99 (C) season.

The survival rate of crawlers collected from twigs was higher than that for crawlers collected from the roots (Fig. 6.1). This difference was significant during all three years ($F_{(1,72)}$=6.122, P=0.016 for 1996/97; $F_{(1,34)}$=7.61, P=0.009 for 1997/98 and $F=_{(1,71)}$=13.029, P<0.001 for 1998/99), indicating that the origin of crawlers collected during late summer and autumn influenced survival or that crawlers collected from twigs are better adapted for settlement on twigs than those from the roots.

In all of the monthly treatments during the 1996/97 experiment survival of the crawlers from the roots on the Granny Smith trees sprayed with Calnitro® was higher than on untreated trees (Fig. 6.1A). These differences were significant ($F_{(1,89)}$=7.591, P=0.007). However, during the 1997/98 season differences in survival of crawlers from roots on sprayed and unsprayed Royal Gala trees (Fig. 6.1B) were not significant ($F_{(1,71)}$=0.20, P=0.6549). In the 1998/99 season the difference between survival of crawlers from the roots on Granny Smith trees treated with Calnitro® or left untreated, bordered on significance ($F_{(1,88)}$=3.92, P=0.05) as more crawlers survived on the untreated trees during November 1998 (Fig. 6.1C) while more crawlers survived on the treated trees during the rest of the season.

Differences in survival between crawlers collected from both the roots and twigs varied in relation to nitrogen, except for the 1996/97 season when most survived on the nitrogen treated trees (Fig. 6.1A).

6.4. Conclusions

The use of a spray containing nitrogen, in this case Calnitro®, stimulated settling of crawlers on Granny Smith trees but not on Royal Gala. Minks and Harrewijn (1987) showed that artificially increased nitrogen content promoted aphid

settlement and development. This may also explain why Asante (1994) found that *E. lanigerum* fecundity and population growth was the highest during spring and the beginning of autumn when nitrogen levels increased naturally in the trees. Many workers found differences in the ability of *E. lanigerum* to colonise different cultivars (Fluke 1930, Crane *et al.* 1936, Underhill & Cox 1938, Knight *et al.* 1962, Giliomee *et al.* 1968, Brown & Schmitt 1994), which could explain the difference found in this study between settlement on Granny Smith and Royal Gala. Sen Gupta & Miles (1975) reported that *E. lanigerum* had a preference for cultivars with higher amounts of soluble nitrogenous compounds in comparable tissues, and appeared to select feeding sites with a low ratio of phenolics to nitrogen. Because the use of nitrogen may favour *E. lanigerum* crawler survival on Granny Smith trees, alternatives to Calnitro® should be tested for their effects on *E. lanigerum*.

Survival of crawlers collected from the roots was high during early spring and the end of summer. *E. lanigerum* migrate up from the roots into the trees during spring and sometimes also at the end of summer or early autumn (Marlatt 1897, Lohrenz 1911, Lal & Singh 1946, Hoyt and Madsen 1960, Nel 1983, Chapter 4). However, the survival of these crawlers during the height of summer in the orchards may be low as a result of the high temperatures (Chapter 3, 4). Blommers (1994) also found that the survival of crawlers was lower at the higher summer temperatures. Therefore, if migration from the roots could be delayed (see Chapter 7) the number of colonies establishing in the trees would be greatly reduced.

Survival of crawlers originating from twigs was also higher during late summer than the survival of crawlers from the roots. This is the time when crawlers are dispersed by wind (Chapter 4). Successful settlement and survival of crawlers will

result in *E. lanigerum* infestations spreading both within orchards and between orchards during this period. Because it is difficult to control *E. lanigerum* colonies that have already formed in the trees the prevention or reduction of the initial colonisation early in the season is important.

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CHAPTER 7

THE EFFECTS OF STEM BARRIERS ON THE COLONISATION OF *ERIOSOMA LANIGERUM* (HAUSMANN) IN APPLE TREES.

7.1 Introduction

Eriosoma lanigerum (Hausmann) is a secondary pest of apples throughout the world (Brown 1986, Asante *et al.* 1993). It can overwinter on the trees in the Western Cape (Georgala 1953) as in other warmer countries (Baker 1915, Lal & Singh 1945, Bodenheimer 1947, Gambrell & Young 1950, Evenhuis 1958 and 1962, Hoyt & Madsen 1960, Carnegie 1963, Mueller *et al.* 1992, Asante *et al.* 1993, Asante 1994 a, b). However, as a result of a number of factors, few or no colonies are visible on the trees during spring. These factors include the physiological condition of the host plant (Thompson 1934, Evenhuis 1962), the appearance of winged females in autumn which have a greatly reduced fecundity (Hely *et al.* 1982, Asante 1994a), parasitism by *Aphelinus mali* (Hald.) in late summer (Bodenheimer 1947, Hoyt & Madsen 1960, Thakur *et al.* 1988) and spring after completion of diapause. Rain and wind that wash or blow colonies from the trees during winter, as well as low temperatures (Schoene & Underhill 1935) also contribute to the decline in colony numbers. In addition, many colonies are removed during winter pruning, while compounds applied at the end of August to combat delayed foliation, *Quadraspidiotus perniciosus* (Comstock) and

Pseudococcus pests on the trees will also serve to reduce the overwintering population of *E. lanigerum* (Georgala 1953, Chapter 4).

Most of the initial colonisation of the aerial parts of the tree is from aphid colonies on the roots (Brown & Schmitt 1994, Chapter 4). In spring and summer large numbers of *E. lanigerum* crawlers move from the roots up into the trees (Theobald 1920, Schoene & Underhill 1935, Madsen & Hoyt 1957, Chapter 4) and settle in the leaf axils where they establish colonies.

The use of trunk barriers for the control of banded fruit weevil, *Phlyctinus callosus* Boh., on apples has been developed (Barnes *et al.* 1994, Barnes *et al.* 1995, Barnes *et al.* 1996) and is used widely in the apple orchards in the Western Cape Province. These barriers reduced the upward migration of *E. lanigerum* crawlers from the roots early in the summer (Barnes *et al.* 1994, Chapter 4). However, wind may also disperse the crawlers (Schoene & Underhill 1935, Greenslade 1936, Hoyt & Madsen 1960, Chapter 4), negating the effect of the barriers. Therefore, the effect of stem barriers on the establishment of *E. lanigerum* colonies on twigs can not be quantified by simply measuring the reduction in upward migration. This study was conducted to determine the effect of two kinds of fruit weevil stem barriers on the colonisation of *E. lanigerum* in the apple trees.

7.2 Material and methods

7.2.1 Sites

A block of four Granny Smith orchards (site one), which had a light infestation of *E. lanigerum*, was used. Each orchard was about 2 ha in size and was

divided into two sections. One randomly selected half of each orchard was banded, using Protector Strip® bands while the other half remained unbanded. The Protector Strip is an 8 cm wide composite of synthetic fibre batting covered with a plastic strip flayed at the bottom edge (Barnes *et al.* 1994).

A heavily infested orchard of about 2 ha (site two) was used on a second farm, also in the Elgin area. Granny Smith and Starking trees were present but sampling was confined to the Granny Smith trees. Half of the orchard was banded using sticky Envirobands and the other half was left unbanded. An Enviroband consisted of a 5 cm wide plastic strip, factory coated with a non-drying glue, Plantex® (Barnes *et al.* 1994).

The experiment was conducted during two years and sampling was carried out every second week from October 1993 until July 1994 in the first season and from October 1994 until 12 April 1995 in the second season. The trees on the second site were grafted at the end of the first season and all the trees were cut down to approximately the same size.

7.2.2 Sampling

7.2.2.1 Crawler movement

Masking tape of which a 5 cm long strip was painted with Plantex® was stuck to the stems of five evenly spaced trees in the blocks. The masking tape was placed above and below the fruit weevil band in the banded blocks. This was done to obtain information on the crawler movement from the roots as well as information on the number of crawlers finally entering the tree by crawling up the trunk. In the unbanded blocks two masking tape strips were also used, one above the other, to simulate the

pattern used in the banded blocks. The masking tape strips were replaced every second week. They were placed with the sticky Plantex® side on transparent plastic and transported to the laboratory where the number of crawlers on each strip was counted using a microscope.

7.2.2.2 *E. lanigerum* colonies in trees

E. lanigerum colonies were counted at two weekly intervals on 25 evenly spaced trees in each block. A colony was considered to be one or more feeding aphids, and where heavy infestations caused colonies to become contiguous, each obvious centre of concentration was regarded as a colony. If more than 2 cm of stem was covered by aphids each 2 cm of covered stem was regarded as a colony. The colonies were only counted on one half of the each of the trees, as it was difficult to manoeuvre through the rows of the trees to count on the other half. A distinction was made between the colonies that contained mummies resulting from parasitism by *A. mali* and colonies that were not parasitised.

7.2.2.3 Aerial movement of crawlers and *A. mali*

Flat yellow sticky traps (15cm by 10 cm) were used to sample the aerial movement of *E. lanigerum* crawlers and *A. mali*. The traps were hung in every second tree used for counting *E. lanigerum* colonies. They were replaced every fourth week and when they were removed they were placed with the sticky side on transparent plastic. The *E. lanigerum* crawlers and adult *A. mali* were counted using a microscope in the laboratory.

7.2.3. Data analysis

The data from site one were analysed as a factorial design. Each of the four orchards was regarded as a block, and the main effects were sampling date and treatment (banded or unbanded). All the data pertaining to counts of colonies, counts of crawlers and *A. mali* on yellow traps were transformed using log (x+1) before analysis to stabilise the variance. On site two the blocks were not replicated. Therefore hypothesis testing could not be conducted and the data were illustrated graphically and used as descriptive support for the data obtained from site one.

7.2.3.1 Crawler movement

The average number of crawlers recorded on the top masking tape strip divided by the average recorded on the bottom strip was used in the analyses. This provided an estimate of the proportion of crawlers entering the tree via the trunk.

7.2.3.2 *E. lanigerum* colonies in trees

The average number of *E. lanigerum* colonies per half tree were used in the analyses.

7.2.3.3 Aerial movement of crawlers and *A. mali*

Data pertaining to the average number of crawlers and the average number of *A. mali* per trap were used in the analyses.

7.3 Results

7.3.1 Crawler movement

At site one more *E. lanigerum* crawlers were recorded moving up the trunk (over time in the trees) during the 1994/95 season than during the 1993/94 season

(Fig. 7.1.), although peak crawler movement was similar in both seasons. There were no interactions between date and treatment (banded and unbanded) or differences between the two treatments in the proportion of crawlers entering the aerial parts of the trees during either season (Table 7.1). A higher proportion of crawlers was found on the top masking tape strip late in the 1994/95 season than during the first half of the season. This was because crawler movement in the trees increased during autumn when *E. lanigerum* numbers in the trees were high (Fig. 7.3), resulting in an increased probability of crawlers being trapped in the sticky masking tape strip.

On site two high numbers of crawlers moved into the trees from October until December during both seasons although more moved up during the 1993/94 than during the 1994/95 season. There were considerably more crawlers on the masking tape strips below the Envirobands than above the bands (Fig. 7.2), suggesting that these fruit weevil bands severely restricted the crawler movement up the trunks. In addition, there was less crawler movement in the section without Envirobands than in the section with the bands (Fig. 7.2), as was seen on the bottom bands. This suggests lower populations on the roots in the unbanded section. In the banded section activity was so high that the Envirobands on some trees were completely covered by crawlers, allowing some crawlers to walk over the bands without getting stuck.

7.3.2 *E. lanigerum* colonies in trees

At site one there were no interactions between date and treatment (banded and unbanded) in the number of colonies found in trees during either season (Table 7.2). There was a difference in the number of colonies between banded and unbanded (Table 7.2) trees during the 1993/94 season, with more colonies in the unbanded trees

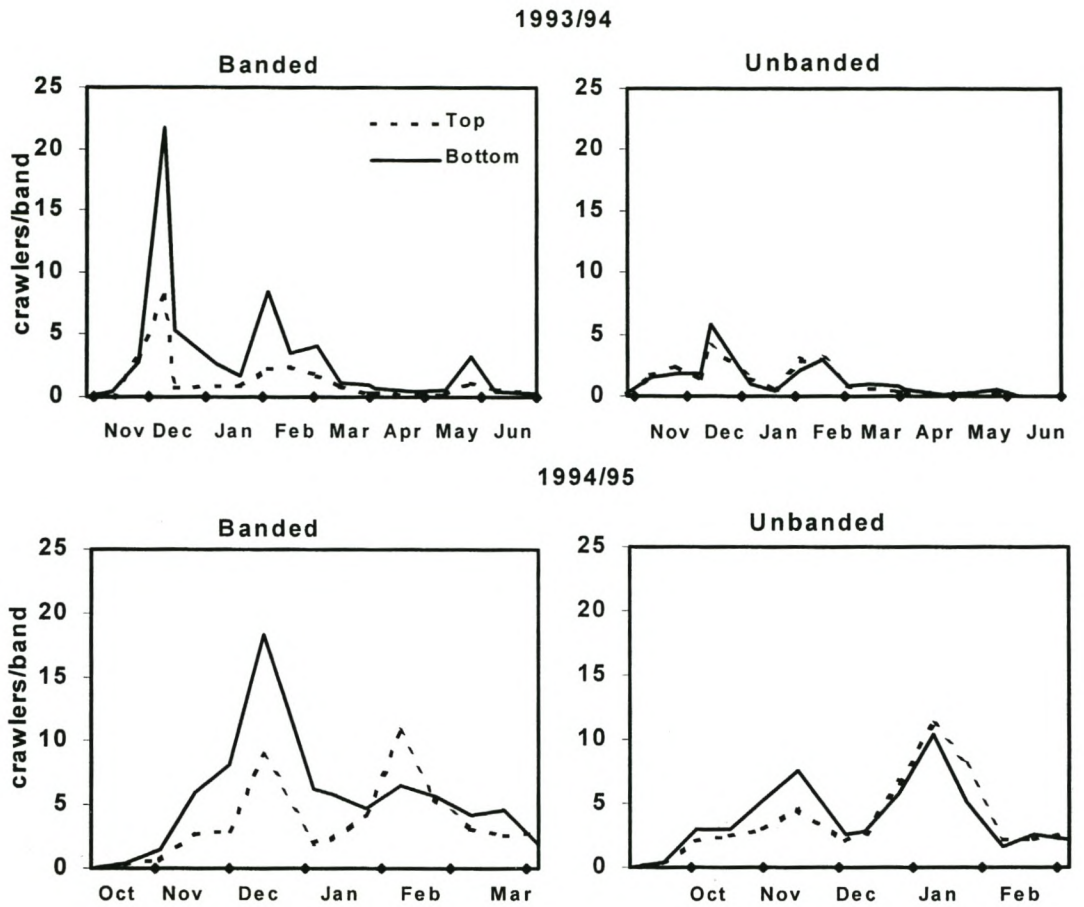

Fig. 7.1. Average number of *Eriosoma lanigerum* crawlers on the bottom bands (Bottom) and on the top bands (Top) for the banded and unbanded blocks on site 1 during the 1993/94 and 1994/95 seasons.

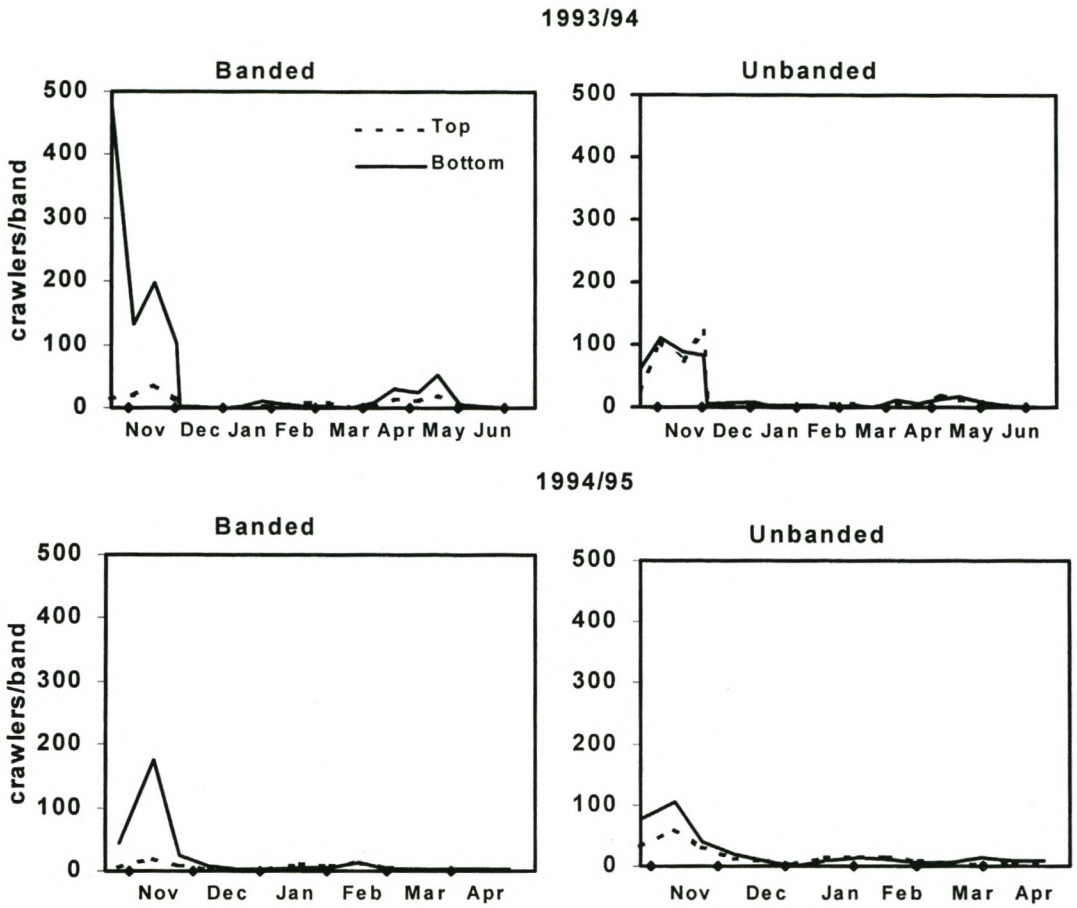

Fig. 7.2. Average number of *Eriosoma lanigerum* crawlers on the bottom bands (Bottom) and on the top bands (Top) for the banded and unbanded block on site 2 during the 1993/94 and 1994/95 seasons.

Table 7.1. Analysis of variance of the proportion of *Eriosoma lanigerum* crawlers entering the trees at site one during the 1993/94 and 1994/95 seasons.

	1993/94 season				1994/95 season			
Source	D.F	Mean Square	F-value	P	D.F	Mean Square	F-value	P
Dates	17	0.061845	2.45	0.003	13	0.069428	2.291	0.012
Treatments	1	0.000122	0.005	0.945	1	0.002119	0.067	0.792
Interactions	17	0.030999	1.228	0.255	13	0.016748	0.553	0.884
Residual	108	0.025239			84	0.030305		

Table 7.2. Analysis of variance of the number of *Eriosoma lanigerum* colonies in the trees at site one during the 1993/94 and 1994/95 seasons.

	1993/94 season				1994/95 season			
Source	D.F	Mean Square	F-value	P	D.F	Mean Square	F-value	P
Dates	18	0.090393	7.70	<0.001	13	1.240568	63.74	<0.001
Treatments	1	0.046144	3.93	0.050	1	0.003256	0.17	0.684
Interactions	18	0.008562	0.73	0.774	13	0.006838	0.35	0.981
Residual	108	0.025239			84	0.019462		

Table 7.3. Analysis of variance of the number of *Eriosoma lanigerum* crawlers on yellow sticky traps on site one during the 1993/94 and 1994/95 seasons.

	1993/94 season				1994/95 season			
Source	D.F	Mean Square	F-value	P	D.F	Mean Square	F-value	P
Dates	7	0.016397	12.71	<0.001	6	0.022918	5.78	<0.001
Treatments	1	8.4×10^{-7}	0.001	0.980	1	0.004408	1.11	0.298
Interactions	7	0.001204	0.933	0.490	6	0.001754	0.44	0.846
Residual	48	0.00129			42	0.003965		

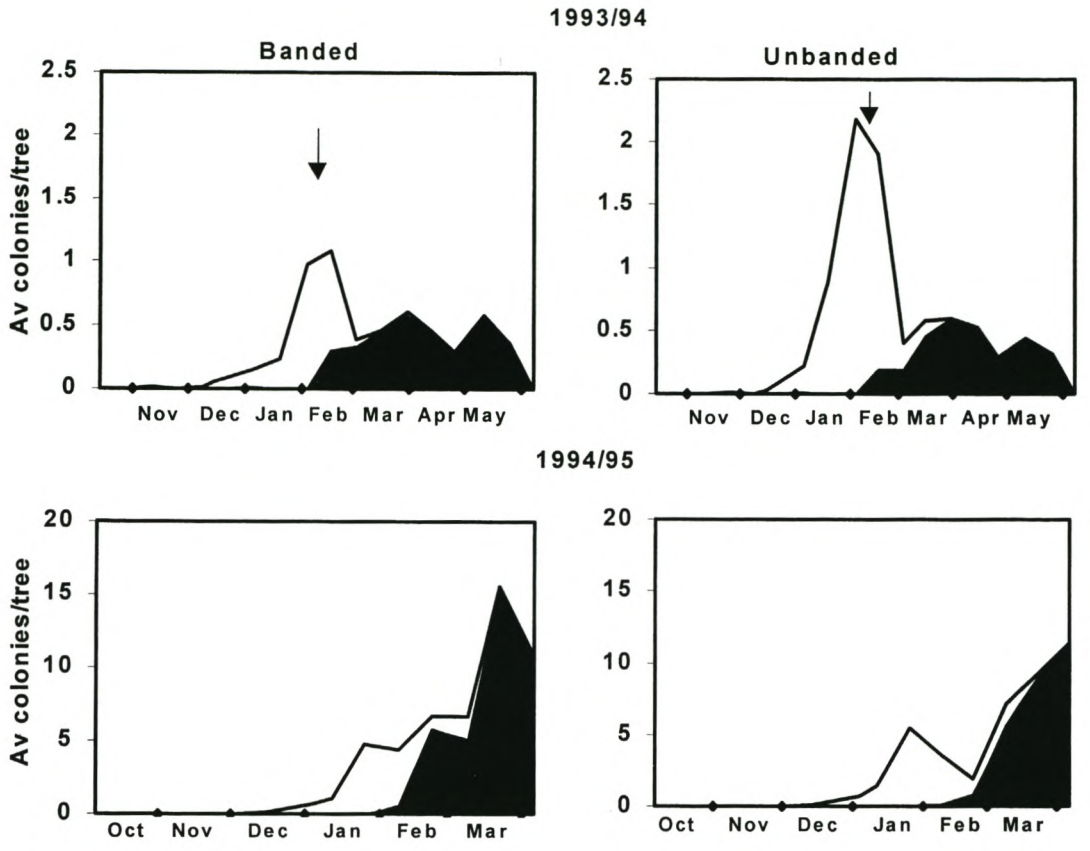

Fig. 7.3. Average (Av) number of *Eriosoma lanigerum* colonies per half tree (line) and the average number of parasitised colonies (area) for the banded and unbanded blocks on site one during the 1993/94 and 1994/95 seasons. The time endosulfan was applied is indicated by an arrow. Note the scale difference between graphs for 1993/94 and 1994/95.

than in the banded trees, particularly during January and February 1994 (Fig. 7.3). An endosulfan spray was applied at the end of February 1994, after which colony numbers dropped in both treatments and remained constant until the end of the season. During the 1994/95 season colony numbers were higher than during the 1993/94 season (Fig. 7.3) but there were no differences between treatments (Table 7.2).

The number of parasitised colonies only started to increase from the second week in February and by the end of March most of the colonies were parasitised (Fig. 7.3). During the second season parasitised colonies appeared during mid January 1995 and increased from then on. However, colony numbers increased even after most of the colonies had been parasitised (Fig. 7.3).

At site two fewer colonies were counted on the banded trees than on the unbanded trees (Fig. 7.4) during the 1993/94 season. However, during the 1994/95 season colony numbers in the two treatments were similar. An endosulfan spray applied on 13 December 1993 at site two caused a temporary decline in colony numbers. Early in January a second endosulfan spray again reduced the aphid population. Peak crawler movement was over before the last spray. Therefore, the large difference in the colony numbers between the two treatments (banded and unbanded) that developed thereafter could not be attributed to the effect of the Envirobands alone. The trees in the unbanded section were very tall and chemicals applied for *E. lanigerum* control did not reach the tops, where most of the colonies were counted after the spray application. This contributed greatly to the differences in colony numbers between the banded and unbanded trees, later in the season. Parasitised colonies were recorded from the end of March 1994 in the first season.

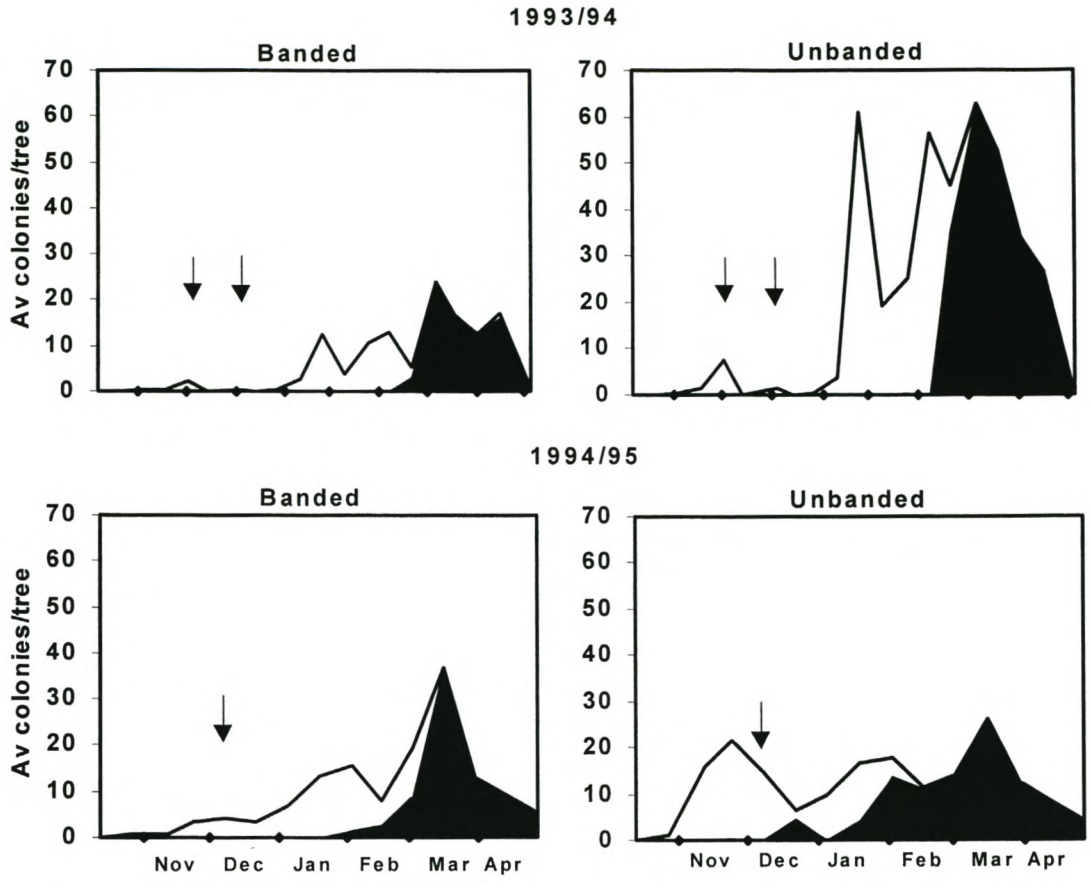

Fig. 7.4. Average (Av) number of *Eriosoma lanigerum* colonies per half tree (line) and the average number of parasitised colonies (area) for the banded and unbanded block on site two during the 1993/94 and 1994/95 seasons. The time endosulfan was applied is indicated with arrows.

From the first week of May 1994 all the colonies were parasitised.

On site two more colonies were recorded early during the second season in the unbanded block than in the banded block (Fig. 7.4). After the endosulfan spray in December 1994 colony numbers increased at the same rate in the two blocks. Wounds where the trees were grafted at the end of the previous season provided refuges enabling aphids to escape contact with the chemical spray and resulting in a continuous residual population in the trees. Therefore, movement from the roots later in the season did not result in a noticeable increase in colony numbers. During the second season parasitised colonies were recorded from early in the season, but numbers only started increasing at the end of January 1995.

7.3.3. Aerial movement of crawlers and *A. mali*

At site one low numbers of *E. lanigerum* crawlers and *A. mali* adults were recorded on the yellow traps during both seasons (Fig. 7.5). There were no interactions between date and treatment (banded and unbanded) or differences between treatments in the number of crawlers found on the yellow traps during either season (Table 7.3). There were differences between dates in the number of *E. lanigerum* crawlers found on the yellow traps during both seasons (Table 7.3). They increased from spring and reached peak numbers during February (Fig. 7.5) when most colonies were recorded in the trees (Fig. 7.3). The numbers declined as *A. mali* activity increased during autumn.

There were no interactions between date and treatment (banded and unbanded) or differences between treatments in the number of *A. mali* on the yellow traps during

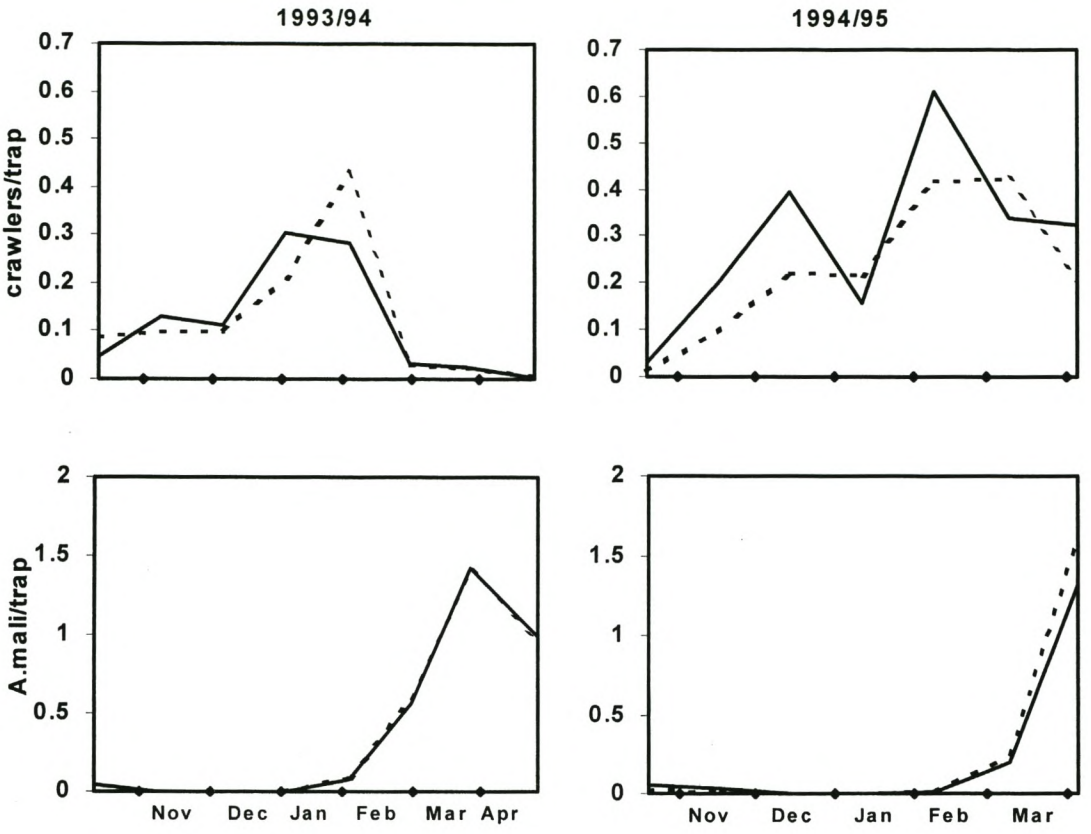

Fig. 7.5. Average number of *Eriosoma lanigerum* crawlers and *Aphelinus mali* adults on yellow sticky traps on site one for the banded (solid line) and unbanded blocks (broken line) during the 1993/94 and 1994/95 seasons.

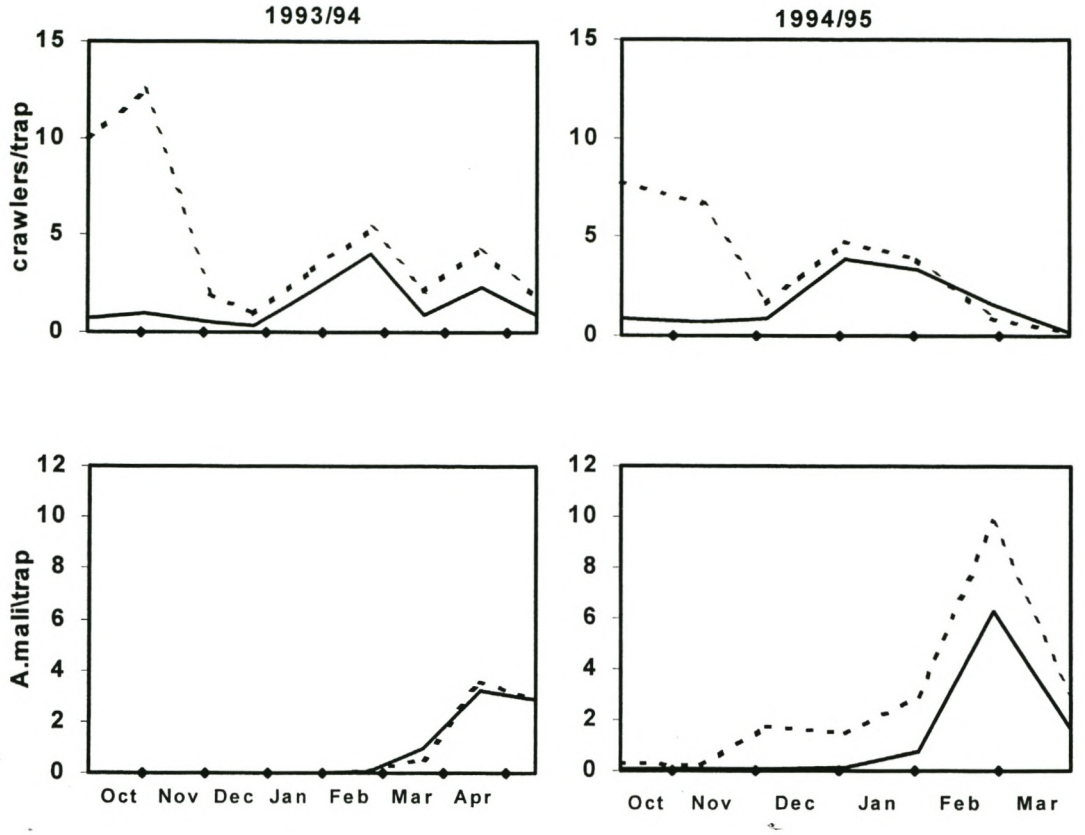

Fig. 7.6. Average number of *Eriosoma lanigerum* crawlers and *Aphelinus mali* adults on yellow sticky traps on site two for the banded (solid line) and unbanded block (broken line) during the 1993/94 and 1994/95 seasons.

either season (Table 7.4). However large differences in *A. mali* numbers between dates were recorded on the yellow traps during both seasons (Table 7.4). A few parasitoids were found early during both seasons (Fig. 7.5) after which they disappeared until the beginning of February. Numbers increased rapidly during the first season but declined after April 1994, probably as a result of low temperatures.

At site two high numbers of crawlers were recorded early during both seasons on the yellow traps in the banded and unbanded blocks (Fig 7.6). This corresponded with high counts on the masking tape strips (Fig. 7.2). The yellow traps in the banded section caught fewer *E. lanigerum* crawlers than those in the unbanded section, particularly early in the season. This reflected lower activity in the banded section. During both seasons crawler numbers on the sticky traps increased in both treatments from the end of January, probably as a result of an increase in the number of colonies in the trees (Fig. 7.4). The result was that from February onwards crawler numbers on the yellow traps were similar in both treatments (Fig. 7.5). Crawler numbers on sticky traps declined after the peak upward movement in the spring as well as when the endosulfan sprays were applied. At the end of the season the numbers decreased when colony numbers declined.

A. mali numbers were relatively low on the sticky traps during the first season (Fig. 7.6) and only started to increase from the end of March 1994. During the second season adults were recorded on the traps from November 1994. Numbers increased from January 1995 until the end of February after which they declined. This coincided with the drop in *E. lanigerum* colony numbers (Fig. 7.4).

Table 7.4. Analysis of variance of the number of *Aphelinus mali* adults on yellow traps at site one during the 1993/94 and 1994/95 seasons.

	1993/94 season				1994/95 season			
Source	D.F	Mean Square	F-value	P	D.F	Mean Square	F-value	P
Dates	7	0.188877	211.34	<0.001	6	0.16413	47.12	<0.001
Treatments	1	0.000192	0.22	0.645	1	0.00014	0.04	0.842
Interactions	7	0.000103	0.12	0.997	6	0.000447	0.13	0.992
Residual	48	0.000894			42	0.003483		

7.4 Discussion

Fruit weevil bands limited crawler movement into the trees. They did not prevent but only delayed colonisation. This could give the natural enemy, *A. mali*, time to recover from the early season spraying and low temperatures, and may improve biological control. The parasitoid is suppressed by insecticides sprayed for the control of codling moth, *Cydia pomonella* (L.) (Newton & List 1952, Georgala 1953, Evenhuis 1959, Croft & Hoyt 1978, El-Haidari & Georgis 1978, Penman & Chapman 1980, Cesar *et al.* 1987). In addition *A. mali* develops more slowly than *E. lanigerum* at lower temperatures (Bodenheimer 1947, Evenhuis 1958, Walker *et al.* 1988, Asante & Danthanarayana 1992) in spring. When populations were low on site one there was a significant reduction in colony numbers in the banded section. However, during the second season when the population was high (Table 7.2: Fig. 7.3), there was no reduction. Therefore, bands only have an effect at low population levels, but not when *E. lanigerum* is a real problem.

For these bands to be effective the trees must be free of *E. lanigerum* at the beginning of the season. The bands must be applied before the start of the spring

migration of the crawlers from the roots into the tree. In addition, the area under the trees must be kept free of weeds, which can be used by crawlers to enter the trees. However, aerial dispersal of crawlers will still be a factor in banded plots.

If bands are applied in newly planted orchards the spread of *E. lanigerum* may be reduced. Brown (1986) found that as an orchard matured, more trees become infested with *E. lanigerum*. By the time an orchard was 25 years old, between 79-100% of the trees may be infested. The first instar (crawlers) is the most important stage for local movement (Madsen & Hoyt 1957, Hoyt & Madsen 1960, Asante *et al.* 1993). When colonies are present in the trees these crawlers may disperse with the wind to other apple trees. After forming colonies aphids which fall to the ground can then infest the roots (Hoyt & Madsen 1960) of small trees. These new root colonies may then again act as a reservoir for aphids in coming seasons (Brown & Schmitt 1994).

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CHAPTER 8

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *APHELINUS MALI* (HALDEMAN) AND ON DIAPAUSING LARVAE KEPT IN COLD STORAGE FOR LATER USE IN BIOLOGICAL CONTROL

8.1. Introduction

Aphelinus mali (Hald.) is an effective biological control agent of *Eriosoma lanigerum* (Hausmann) (Sproul 1981, Rawat & Pawar 1987, Mueller *et al.* 1992) in different countries throughout the world. However, in certain areas the control of *E. lanigerum* by *A. mali* alone is not satisfactory. A number of factors may contribute to this, such as cold and dry conditions, high altitude etc. (Lal & Singh 1945, Bodenheimer 1947, Evenhuis 1958, Rawat *et al.* 1989) as well as the use of chemical sprays (Bengston 1960, Lower 1968, Hely *et al.* 1982).

A. mali was imported into South Africa from America in the early 1920s and dispersed quickly. By September 1939 biological control was so effective that chemical control was unnecessary (Lundie 1939). The introduction of DDT for the control of codling moth (*Cydia pomonella* (L.)) in the Western Cape has been given as a reason for

the poor control of *E. lanigerum* by the parasitoid (Georgala 1953) after excellent control was achieved in previous years. However, the degree of control may also be associated with the climatic conditions (DeBach 1964). In areas where *A. mali* has not performed satisfactorily, it appeared as if there was a lag between parasitoid activity and that of the aphid, resulting from the differential temperature thresholds for development and reproduction between the host and its parasitoid (Bodenheimer 1947, Lung *et al.* 1960, Bonnemaison 1965).

E. lanigerum is becoming more important as a pest of apples in the Elgin area. In addition, *E. lanigerum* populations are developing resistance to chemicals used for commercial control (Pringle *et al.* 1994). Therefore, it is important to find or enhance other means of control.

Recent studies have indicated that both *E. lanigerum* and *A. mali* are active throughout the winter (Chapter 4). However, a certain proportion of the *A. mali* population enters diapause and emerges at the end of winter and early spring when they eliminate most of the remaining *E. lanigerum* colonies. They then disappear due to a lack of food. Subsequent to this, migration of *E. lanigerum* crawlers from the roots to the aerial parts of the trees commences and colonies establish in the trees when parasitoids are not present in sufficient numbers to control them. Early season sprays against the other important pests, such as codling moth, could further adversely affect the remaining few parasitoids (Bengston 1960, Lower 1968, Hely *et al.* 1982, Chapter 10).

There are conflicting reports on the relative rates of development of *A. mali* (Telenga 1935, Bodenheimer 1947, Evenhuis 1958, Bonnemaison 1965, Walker *et. al.* 1988, Asante & Danthanarayana 1992). Temperature is one of the most important

physical factors known to have a differential effect on the development of aphids and their parasitoids (Campbell *et al.* 1974). The effects of temperature on *A. mali* in South Africa have not been studied. Therefore, the influence of different constant temperatures on the development of local *A. mali* was investigated.

The possibility of extending diapause of *A. mali* by keeping twigs bearing mummies in cold storage was investigated as this could be used by producers to ensure that parasitoids are available when colonies establish in the trees after migrating from the roots in spring (Chapter 4). This would greatly improve biological control of *E. lanigerum*. In this study the survival of mummies in cold storage after being collected early in the winter was determined, as well as the optimum time at which twigs should be removed from orchards. The expected developmental rate of parasitoids at different temperatures after being held in cold storage was also determined. This would assist in the timing of placing mummies in the orchard so that they would emerge when the upward movement of *E. lanigerum* crawlers started.

8.2. Material and methods

8.2.1. The influence of constant temperature on *A. mali* development

E. lanigerum colonies were established on small potted apple (Granny Smith seedling) trees (not higher than 15 cm). The seedlings were enclosed with transparent plastic containers (16 cm high, 8 cm in diameter) covered on top with fine gauze. Between 2 and 5 mated female *A. mali* and a few males were introduced into each container for the parasitisation of *E. lanigerum*. Cotton wool soaked in a 40% sugar

solution was placed in a plastic cap at the base of each tree as a food source for the wasps.

The trees were kept in incubators with temperatures set at 15, 20, 25, 27 and 30°C, a humidity range of 80-90% and a 16:8 (L:D) photoperiod. After 24 hours all the wasps were removed and the trees returned to the incubators at the different temperatures. The trees were examined every 24 hours for the appearance of black mummified woolly apple aphids, which indicated that larval development was complete (Lundie 1924). The mummies were then removed and placed into small glass vials (5 cm long and 1.5 cm diam) plugged with cotton wool. The mummies inside the vials were returned to the incubators. They were examined every 24 hours for the emergence of *A. mali* adults. The time to the formation of mummies, the time from formation of mummies until emergence and the sex of the wasps were noted.

The reciprocal of time in days to complete development from egg to the formation of the mummies (larval development) and for the time to complete development from mummy formation to the appearance of the adult (development of pupae) were regressed on temperature. The minimum temperature for larval and pupal development was estimated by solving the regression of 1/Time=0 (Campbell *et al.* 1974). Differences in the regression lines for development from mummified aphid to the appearance of the female and the male wasps were examined using dummy variables (Gujarati 1970). The number of degree days (°D) required for development of *A. mali* was calculated using °D=1/b, where b is the slope of the regression of 1/Time on temperature (Campbell *et al.* 1974).

8.2.2. Survival in cold storage

Twigs bearing *E. lanigerum* mummies were collected from the farm Glenbrae (34.14 S 19.9 E) in the Elgin district during the winter (10 June 1992), and were kept in a dark cold store at approximately 1-4°C. Twigs were removed from cold storage at weekly intervals until the second week of October. The mummies were removed from the twigs and placed individually into gelatine capsules. Fifty of these capsules were put into a petri dish as described by Trimble *et al.* (1990). There were four replicated petri dishes each week to give a total of 200 mummies. These mummies were then placed in an incubator set at a temperature of 25±1°C, and a L:D cycle of 16:8 hours.

In addition, during 1992 on 18 June, 2 July, 15 July, 5 August, 26 August and 9 September twigs bearing mummies were brought from Glenbrae on the first three dates and thereafter from Oak Valley (34.9 S 19.2 E) because the apple trees on Glenbrae were pruned, making it difficult to find mummies on this site. From these twigs 200 mummies were placed in gelatine capsules as described above. These were placed directly into the incubator.

All the mummies were examined every 24 hours for emergence of adult wasps after being placed in the incubator at 25°C. The time to emergence and the sex were recorded. The emergence of other parasitoid species and hyperparasitoids was also noted. From 15 October onward the mummies were dissected if no parasitoids appeared after 60 days and the developmental stage was noted if possible. Adult parasitoids that appeared within 7 days at 25°C were assumed to have been actively growing inside the mummy and therefore not in diapause or postdiapause. (see 8.3. Results. 8.3.1. The influence of constant temperatures on *A. mali* development).

8.2.3. Optimum time for collecting mummies

Apple twigs with *E. lanigerum* mummies were collected from Oak Valley on 16 June, 14 July and 11 August 1993. The twigs were stored at 1-4°C. From 17 July 1993 200 mummies were treated every second week as described above and placed in the incubator described above at 25°C ±1. The gelatine capsules with mummies were examined every day for the emergence of parasitoids and their sex was determined. Total emergence of *A. mali* from mummies collected on the three dates was compared using an analysis of variance. To determine the numerically dominant life stage present at the start of the experiment 100 mummies were dissected after they had been collected from the orchard. This could not be done on the last collection date, as sufficient mummies could not be found. On 14 July twigs were also collected from a second site Dennebos, in the Vyeboom area and treated as described above. This was done to determine whether or not there were regional differences. The difference between survival of the diapausing *A. mali* from the two areas was tested as described by Cox (1970).

8.2.4. Postdiapause development at different temperatures

Apple twigs with parasitised *E. lanigerum* were collected on 15 June 1994 from Oak Valley and kept in a cool room at a temperature of 1-4°C. The day after mummies were collected, 200 were dissected to determine the percentage of each stage present. Four petri dishes, each with 50 mummies in gelatine capsules, were placed in an incubator at 25°C at weekly intervals after August to determine when diapause development came to an end. Capsules were examined daily to determine emergence of

adult parasitoids. All parasitoids were considered to be in postdiapause when additional periods of exposure to cold storage did not result in a noticeable decrease in mean time to emergence (Trimble, 1983).

When diapause was completed, 200 mummies were again dissected to determine survival and the percentage of each developmental stage still alive. Four petri dishes each with 50 mummies in gelatine capsules were placed at 15°C, 20°C, 25°C and 27°C. The capsules were examined daily until adult emergence ceased. This was repeated three times, on 22 September, 12 October and on 8 November 1994.

An analysis of variance was used to test for differences in mean postdiapause developmental time between sexes at the above four temperatures as well as for differences between the three replicates. Linear regression (Campbell *et al.* 1974) was used to estimate the minimum temperature for postdiapause development, t_0. Differences in the regression lines of 1/Time on temperature between males and females was examined using dummy variables (Gujarati 1970). In the full model separate slopes and intercepts were assumed for males and females, while in the reduced model a common slope and intercept was assumed.

8.3. Results

8.3.1. The influence of constant temperatures on *A. mali* development

The times for larval development at the different temperatures are given in Table 8.1.1. Mummified aphids appeared over a long period at each temperature (Fig. 8.1.1). This is not unusual as a variation of as much as 13 days at Ithaca in the U.S.A. (Lundie

1924) or 20 days in the Hood River Valley, Oregon (Childs & Gillespie 1932) have been recorded among individuals reared under the same conditions of temperature and moisture. All life stages can be parasitised but the third instar is the preferred stage (Bodenheimer 1947, Mueller *et al.* 1992). In addition, development in the first and second instars was slower in the Netherlands than in the third and fourth instars (Mueller *et al.* 1992) and parasitoids in wandering woolly apple aphids had greatly increased egg and larval developmental times in Washington (Walker *et al.* 1988). Therefore, the long time interval during which mummies appeared at a given temperature can be explained by the age and activity levels of aphids attacked.

Development of the larval stage was slightly slower at 30°C than at 27 °C (Table 8.1.1). However, the *E. lanigerum* hosts reared at 30°C were under stress resulting in slower development and early death of the aphids (Chapter 3, Marcovitch 1934, Walker *et al.* 1988, Bo & Rongping 1989), and the parasitoid may have been influenced by this negative effect on its host.

Table 8.1.1. Average developmental time from egg to mummy formation (±SD) of *Aphelinus mali* at five constant temperatures.

Temperature (°C)	Developmental time (±SD)	Number used
15	22.17 (±1.58)	204
20	15.22 (±1.13)	110
25	9.60 (±2.82)	217
27	7.21 (±4.42)	92
30	8.27 (±2.89)	207

The regression of rate of development (1/Time) on temperature for larval development fitted well (Fig. 8.1.1; $F_{(1,828)}$=1662,8; P<0.001; R^2=0.67). From this regression a minimum threshold temperature for development (t_0) of 6.72°C was estimated for development from egg to the formation of the mummified aphid. In addition 172.41 °D were required for development. The estimated minimum threshold temperature (6.72°C) compares favourably with the 6.4°C found for egg and larval development of *A. mali* in Washington (Walker *et al.* 1988) and the 6-7°C in Russia (Telenga 1935 in Hagen & Van den Bosch 1968). Although Evenhuis (1958) in the Netherlands reported a minimum temperature threshold of approximately 12°C, the regression for his results gave 10.69°C as the minimum temperature threshold for the development of the eggs and larvae. This is higher than the findings of the present study. However, in both Washington (Walker *et al.* 1988) and the Netherlands (Evenhuis 1958), larval development was more rapid than in our study at all temperatures.

In contrast to the developmental time from egg to mummy formation (Table 8.1.1) the developmental time of the parasitoid inside the mummified aphid was not negatively influenced by the high temperature of 30°C (Table 8.1.2) as it still decreased at 30°C.

The regression lines of developmental rate versus temperature for the time from mummy formation to the emergence of the adult for males and females were not significantly different (F= 0.712; P=0.528). Therefore the data were pooled (Fig. 8.1.2). The regression of the rate of development (1/time) on temperature for pupal development fitted well (Fig. 8.1.2.; $F_{(1,627)}$=2766.75; P<0.001; R^2=0.82).

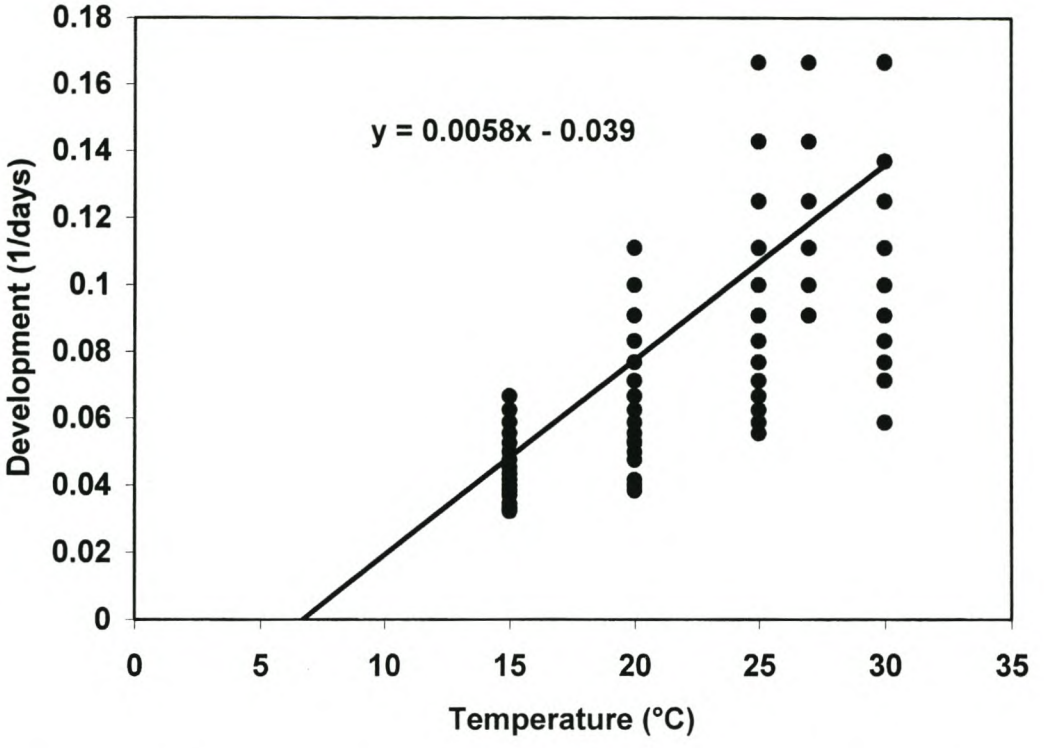

Fig. 8.1.1. Developmental rate (1/days) of *Aphelinus mali* larvae at five constant temperatures.

Table 8.1.2. Mean developmental time (±SD) for *Aphelinus mali* from mummy formation to the emergence of the adult at five constant temperatures.

Temperature (°C)	Females		Males		Pooled data for Females and Males	
	N	Days (±SD)	N	Days (±SD)	N	Days (±SD)
15	93	24.62 (±2.12)	61	24.64 (±2.39)	154	24.63(±2.23)
20	26	12.08(±1.44)	49	8.92 (±4.59)	75	11.48(±1.60)
25	109	7.38 (±0.998)	83	7.43 (±0.87)	192	7.40 (±0.94)
27	21	6.57 (±0.68)	29	5.79 (±1.15)	50	6.12 (±1.04)
30	100	5.93 (±0.90)	60	5.95 (±0.96)	160	5.94 (±0.92)

From the pooled regression of developmental rate on temperature for pupal development (mummy to adult) a minimum threshold temperature for development of 10.27°C was estimated. From the inverse of the slope of the regression an estimated 109.89 degree days were required for the development of the pupae. In Washington the estimated minimum temperature for pupal development was 9.4°C (Walker *et al.*1988) and in Russia, 13°C (Telenga 1935 in Hagen & Van den Bosch 1968). When the inverse of the developmental time of the pupae from the Netherlands determined by Evenhuis

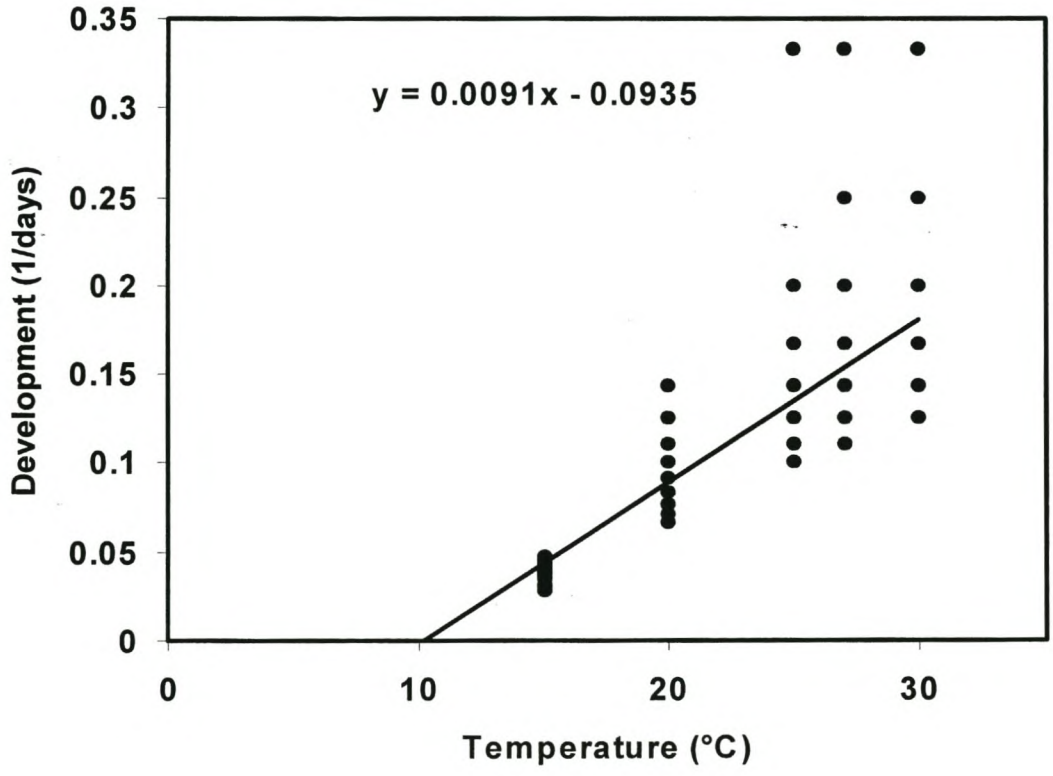

Fig. 8.1.2. Developmental rate of *Aphelinus mali* from mummy formation to adult emergence at five constant temperatures.

(1958) was regressed on temperature a minimum threshold temperature of 5.64 °C was estimated. However, when his results for 30°C were excluded from the regression a value of 9.47°C was estimated for pupal development. Trimble *et al.* (1990) also estimated a minimum developmental temperature of 9.4°C for postdiapause *A. mali* larvae inside the mummy. Although the minimum developmental time was higher in the present study than that found by others, the parasitoids studied here emerged earlier at 30°C than those from the Netherlands (Evenhuis 1958) and Washington (Walker *et al.* 1988). This indicated that the local *A. mali* is better adapted to high temperatures than those from other countries.

8.3.2. Survival in cold storage

Non-diapausing wasps emerged from mummies kept at 25°C for 7 days (see 8.3.1. The influence of constant temperature on *A. mali* development; Table 8.1.2). Therefore, in this section wasps emerging from mummies within a period of up to 7 days at 25°C were assumed to be non-diapausing. Those that emerged from mummies after 7 days at 25°C were assumed to be in diapause.

Mummies collected on the 10th of June and held in cold storage for increasing periods of time produced wasps within 4 days at 25°C after being held in cold storage for up to 8 weeks (Fig. 8.2.1A). These wasps were probably in the pupal and/or adult stage at the time they were collected. A number of wasps also emerged between 5 and 7 days at 25°C (Fig. 8.2.1B) after being held in cold storage for up to 12 weeks. These were assumed to be in the larval stage, but not in diapause or postdiapause at the time of collection.

The low number of wasps emerging within 7 days at 25°C after being held in cold storage for more than 12 weeks (Fig. 8.2.1A and B) suggested that non-diapausing individuals in the mummies did not survive the extended periods in cold storage. Large numbers of wasps emerged from mummies held at 25°C for 8 to 11 days after being held in cold storage between 12 and 17 weeks (Fig. 8.2.1C). These were assumed to be in diapause at the time of collection. However, as a result of the extended period of cold storage diapause development had been completed. A few wasps continued to emerge from mummies kept at 25°C for more than 11 days (Fig. 8.2.1D) suggesting that diapause was nearly completed and the adults appeared soon after postdiapause development was completed.

There was almost 50% emergence from mummies which had been held in cold storage for 1 to 2 weeks (Fig. 8.2.1A and B). After 17 weeks in cold storage adults emerged from 32.5% of the mummies (Fig. 8.2.1.C and D). Therefore, diapausing and postdiapausing *A. mali* survived cold storage for up to 17 weeks. This would be a sufficient period for survival of mummies on prunings for placing into the orchard during the following spring to augment biological control of *E. lanigerum* early in the season.

If it is assumed that 50% of those that died (did not emerge) (Fig 8.2.1B) after 11 weeks in cold storage were non-diapausing *A. mali,* the rest were diapausing or postdiapausing larvae. Therefore, emergence from the remaining diapausing or postdiapausing larvae collected on 10th of June was as high as 65%, although some could have contained parasitoids that died before the mummies were collected.

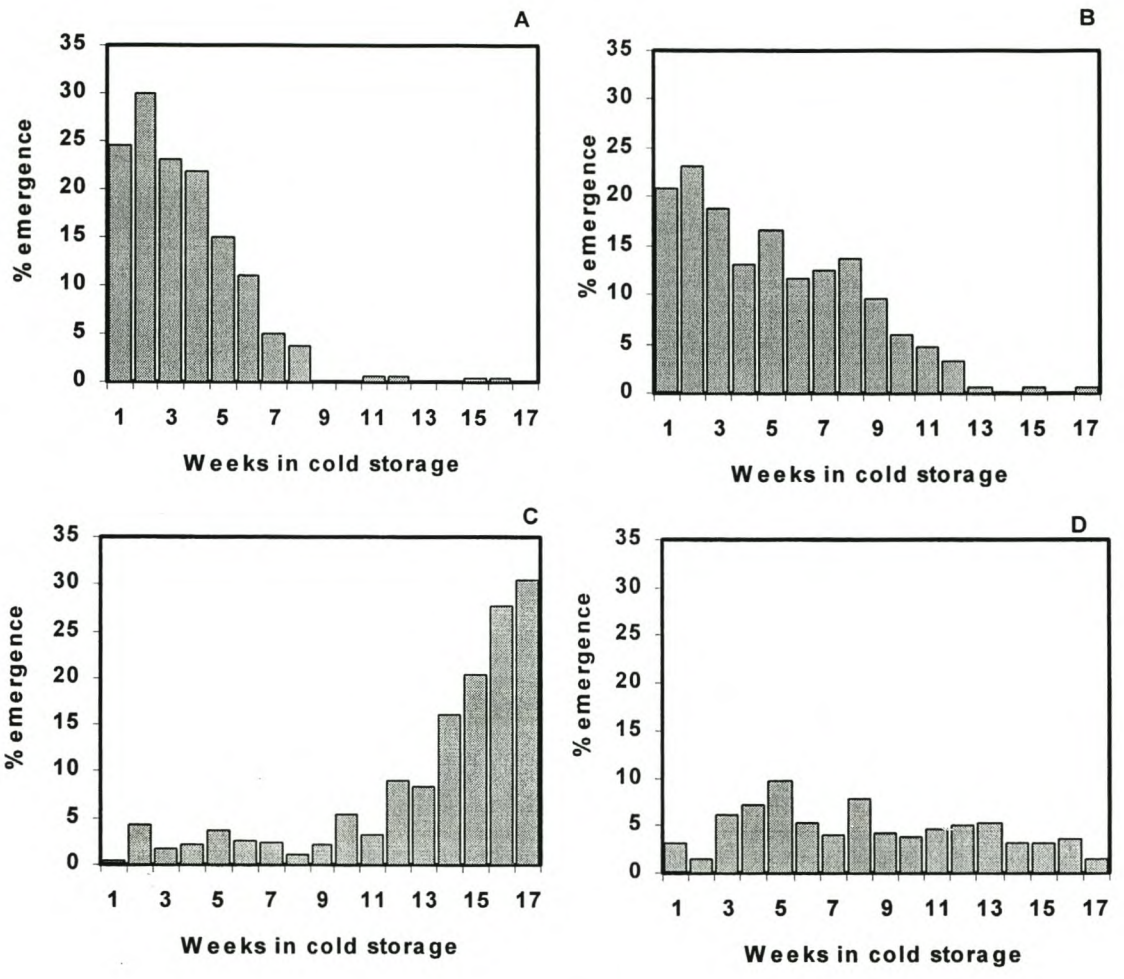

Fig. 8.2.1. Per cent emergence of *Aphelinus mali* from mummies collected on the 10th of June after 4 days (A), between 5 and 7 days (B), between 8 and 11 days (C) and after 11 days (D) at 25°C after being held in cold storage.

The percentage mortality of each lifestage found inside unemerged dissected mummies collected on the 10th of June is given in Fig. 8.2.2. Most died in the larval stage (Fig. 8.2.2A), presumably when they were removed from cold storage before diapause was completed or larvae not in diapause prior to being placed in cold storage where they died. The longer mummies were kept in cold storage the higher was the percentage of dead, partially developed adults (Fig. 8.2.2C). This indicated that adults in mummies could not withstand long periods of low temperatures. Some of the mortality could have been from parasitoids that were near to completion of diapause, but were subjected to postdiapause conditions (25°C) too early, as Tauber & Tauber (1976) reported that high temperatures can result in depletion of metabolic reserves, thereby reducing postdiapause survival. The highest number of dead adults was recorded when parasitoids were held in cold storage for between 9 and 11 weeks. These could have been from mummies containing adults or partially mature adults when the material was collected. These adults could not withstand the long period of cold storage. They could also have been from postdiapausing larvae with depleted energy resources before development could be completed (Tauber & Tauber 1976).

Most of the *A. mali* adults emerged within 7 days from mummies collected on the 18th of June (Fig. 8.2.3), indicating that not all the mummies contained larvae in diapause or postdiapause. More adults emerged within 7 days from mummies collected on the 2nd and 15th of July than from those on the 18th of June (Fig. 8.2.3) suggesting that some of those collected on the former two dates might have completed diapause. The low percentage emergence from mummies collected on the 5th of August may be explained by

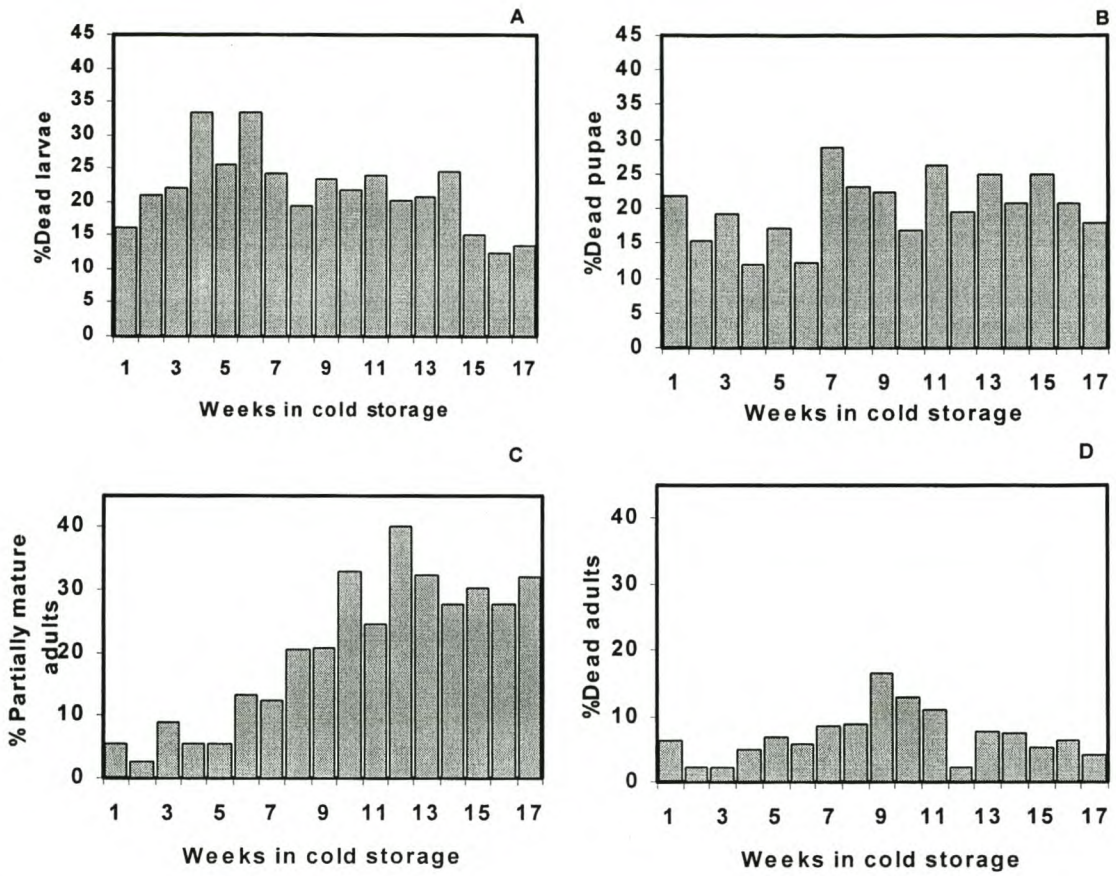

Fig. 8.2.2. Per cent dead *Aphelinus mali* larvae (A), dead pupae (B), dead but partially developed adults (C), and dead, fully developed adults (D) found in dissected mummies collected on the 10th of June and held in cold storage for up to 17 weeks.

the low number of mummies available in the orchard at this time of the year. Most of the mummies on the twigs were empty, as postdiapause development was completed. The remaining mummies probably contained a large number of parasitoids, which died earlier during the winter (Fig. 8.2.3).

Emergence from mummies collected on the 26th of August and the 9th of September was high (Fig. 8.2.3). Those that emerged within 7 days were from non-diapausing parasitoids and the percentage emergence increased. It is possible that these individuals were from *A. mali* adults, which had completed diapause, emerged and were parasitising developing *E. lanigerum* colonies in the trees. Therefore, survival and the total percentage emerging from mummies collected on the 26th of August and the 9th of September was higher than from those collected on the 6th of August (Fig. 8.2.3). A few adults still appeared after 11 days. These may have been from parasitoids which were still in diapause or in the postdiapause stage at the time of collection. Therefore, it would appear as if diapause was mostly completed by the end of August. During September the *E. lanigerum* colonies overwintering in the trees were parasitised by newly emerged parasitoids.

Hyperparasitoids emerged from mummies collected from Glenbrae on four dates between the 10th of June and 15th of July. The percentage of hyperparasitism was 0.5%, 2.9%, 8% and 9% on these dates respectively. Only one hyperparasitoid emerged from the 150 mummies that were collected form Oak Valley during September. The hyperparasitoids were *Aphidencyrtus africanus* Gahan (Encyrtidae), known as a hyperparasite of citrus psylla, and a *Pachyneuron* species (Pteromalidae). Members of

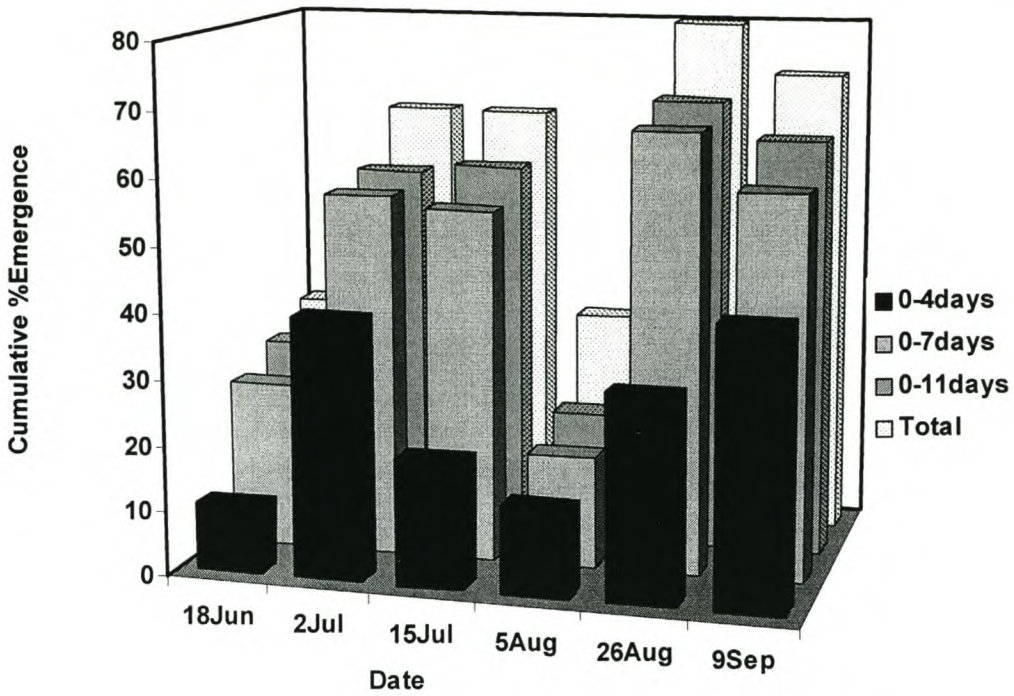

Fig. 8.2.3. The percentage emergence of *Aphelinus mali* after 4, 7, 11 days and the total for mummies placed at 25°C directly after collection from the orchard at different dates.

this genus are often hyperparasites of aphids, coccids and psyllids (Prinsloo, per. comm.). *A. africanus* was the most abundant.

8.3.3. Optimum time for collecting mummies

Mummies collected on the 16th of June and held in cold storage for 6 or more weeks produced large numbers of *A. mali* adults between 8 and 11 days after being placed at 25°C (Fig. 8.3.1C). This suggested that at least 6 weeks of chilling was required to break diapause of *A. mali* collected in mummies at that time. The low numbers which

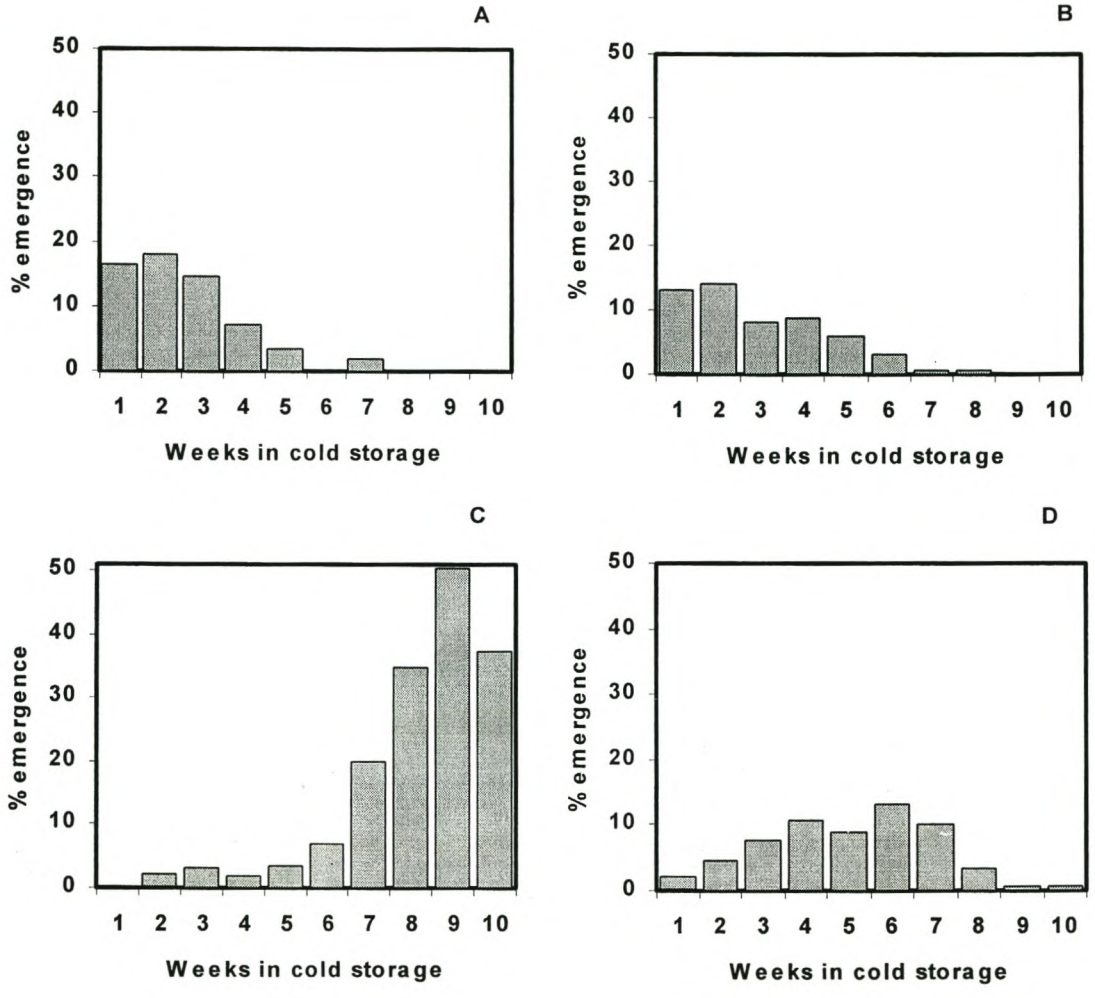

Fig. 8.3.1. Percentage of *Aphelinus mali* adults emerging after 4 days (A), between 5 and 7 days (B), between 8 and 11 days (C) and after 11 days (D) at 25°C, from mummies collected on Oak Valley on 16 June 1993 and kept for various periods in cold storage.

appeared within 7 days (Figs. 8.3.1A and B) indicated that there were some live non-diapausing mature larvae, pupae and adults present in the mummies. The parasitoids still in diapause appeared after 11 days after being placed at 25°C. These declined after being kept in cold storage for 6 weeks as diapause was completed (Fig. 8.3.1D).

Mummies collected on the 14th of July and the 11th of August 1993 on Oak Valley contained more than 30% parasitoids that were non-diapausing as the parasitoids kept for 1-3 weeks in cold storage emerged within 7 days at 25°C (Fig. 8.3.2A and B, and Fig. 8.3.3A and B). The longer the mummies were stored the more adults emerged between 8 and 11 days after being transfered to 25°C, indicating that diapause had been broken (Fig. 8.3.2C and Fig. 8.3.3C). This was also supported by the decline in the number of parasitoids that appeared after 11 days (Fig. 8.3.2D and Fig. 8.3.3D).

The most important consideration determining when mummies should be collected for cold storage is the total percentage emergence after being kept in cold storage. However, there was no significant difference ($F_{2,6}$=1.929; P=0.259) between the percentage adults emerging from material collected in June, July and August from Oak Valley (Fig. 8.3.4). Emergence of *A. mali* from mummies when removed from cold storage on 21 October was 38%, 40% and 32% for those collected during June, July and August respectively. Therefore, other factors such as the availability of mummies must also be considered. Mummies are more plentiful during early winter (June and July) than later as rain and wind can dislodge mummies from the branches or damage them (Chapter 4, Lung *et al.* 1960). It would, therefore, be advisable to collect mummies during June and July. In addition after August most orchards are sprayed with a winter oil and lime

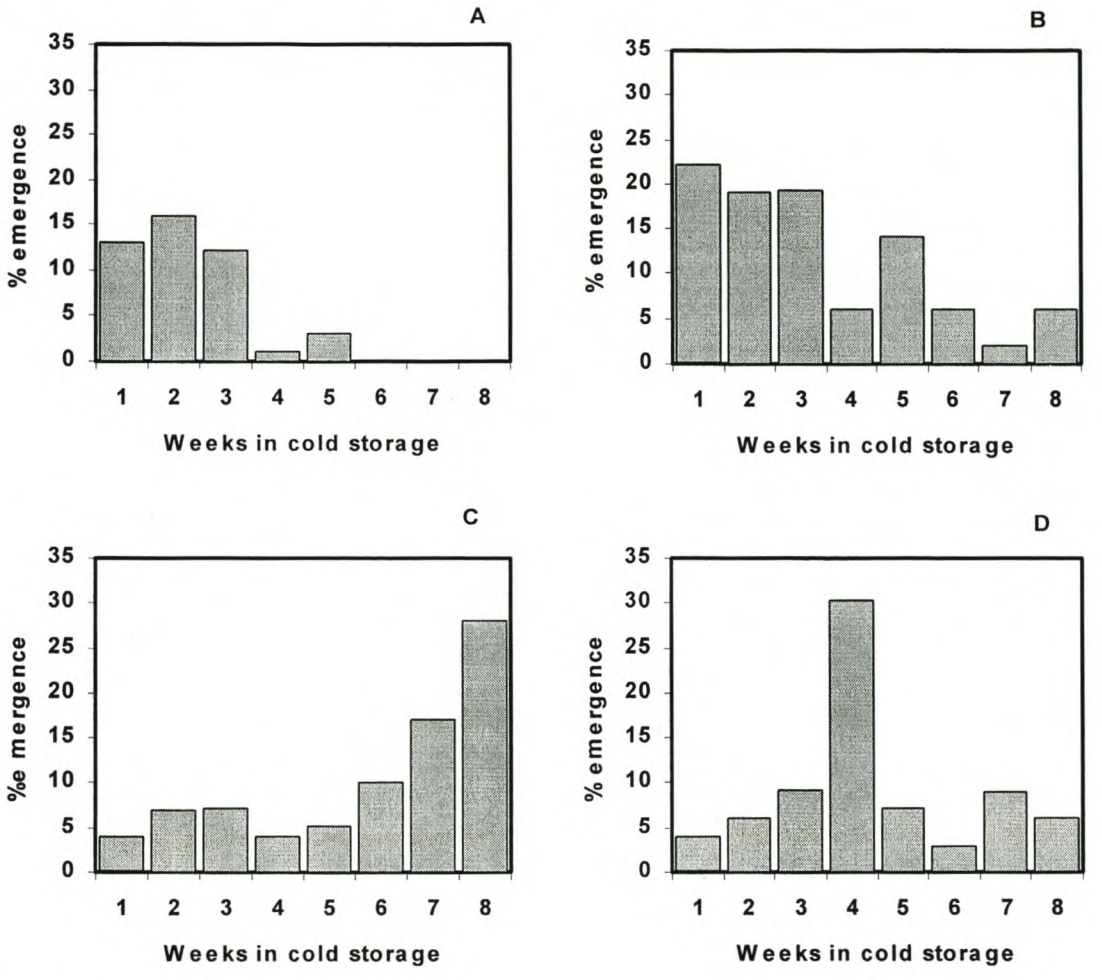

Fig. 8.3.2. Percentage of *Aphelinus mali* adults emerging after 4 days (A), between 5 and 7 days (B), between 8 and 11 days (C) and after 11 days (D), at 25°C from mummies collected on Oak Valley on 14 July 1993 and kept for various periods in cold storage.

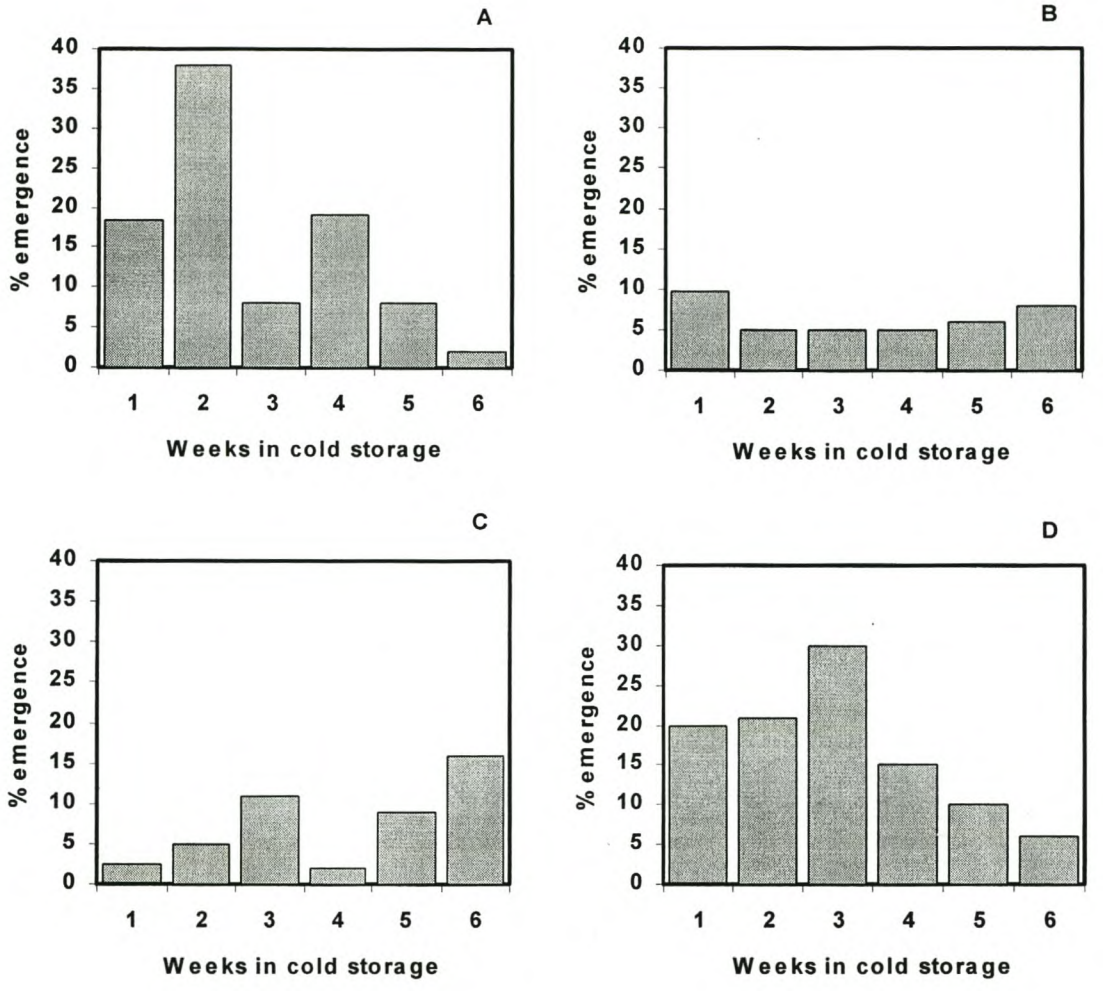

Fig. 8.3.3. Percentage of *Aphelinus mali* emerging after 4 days (A), between 5 and 7 days (B), between 8 and 11 days (C) and after 11 days (D) at 25°C, from mummies collected on Oak Valley on 11 August 1993 and kept for various periods in cold storage.

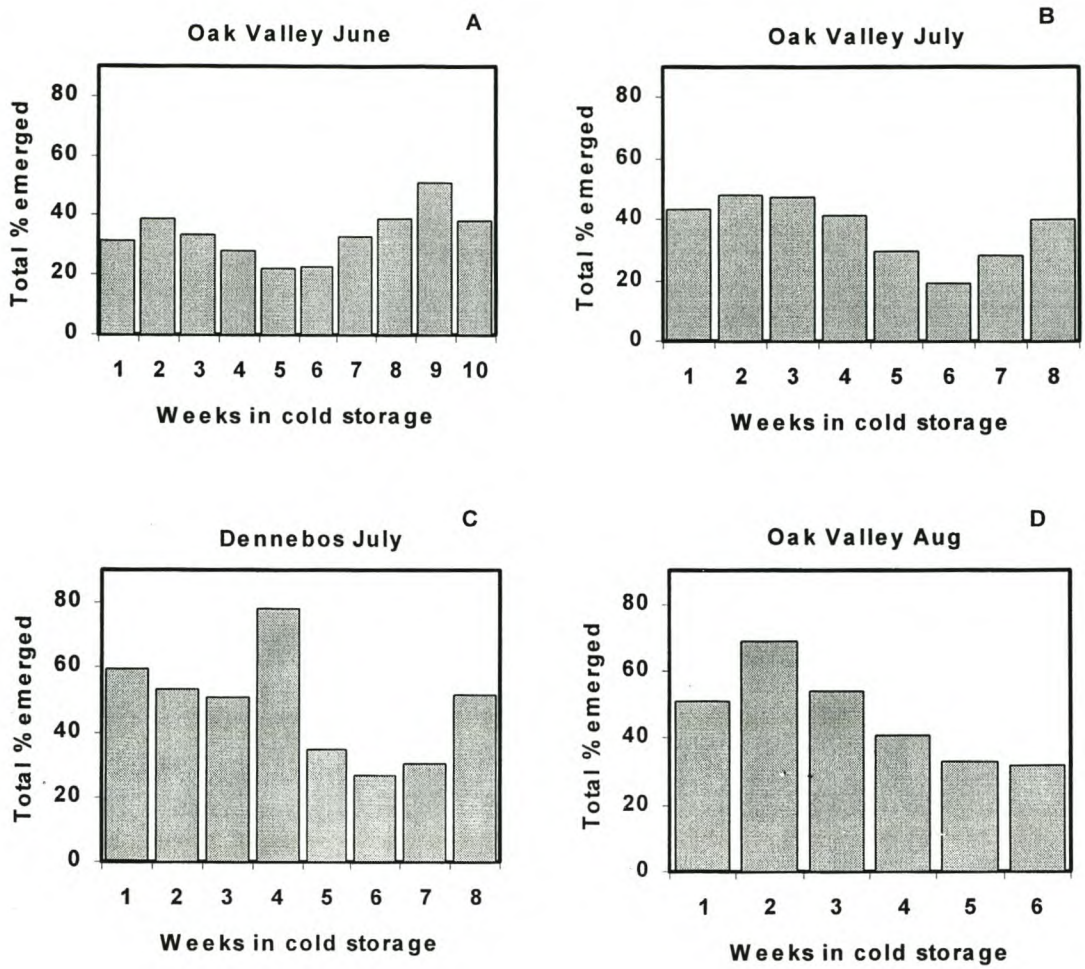

Fig. 8.3.4. Total percentage emergence of *Aphelinus mali* from mummies collected on Oak Valley in June (A), July (B), and on Dennebos in July (C) as well as on Oak Valley in August (D) when placed at 25°C after cold storage.

sulphur or oil and DNOC to combat delayed foliation, *Quadraspidiotus perniciosus* (Comstock) and any hibernating insect pests on the trees (Georgala 1953). These sprays kill the rest of the mummies.

Postdiapausing mummies kept at 25°C from 21 October, appeared 8 to 11 days later (Fig. 8.3.1C, 8.3.2C and 8.3.3C). This indicated that these adults would appear at the end of October when *E. lanigerum* crawlers entered the aerial parts of the apple trees from the roots in large numbers each year. However, as the temperature in the orchard is not constant at 25°C as it is in the growth chamber the time of emergence will differ.

Dissection of mummified aphids collected on Oak Valley during June before being placed in cold storage showed that 8% of the individuals were already dead (Table 8.3.1). Most of the live parasitoids were in the larval stage followed by pupae and the pre-imaginal stage. As the final instar larva is the diapausing stage of *A. mali* (Lundie 1939, Evenhuis 1958) the pupae, pre-imaginal wasps and adults inside the mummies were from non-diapausing *A. mali.* They were part of the 50% of parasitoids which do not enter diapause during winter (see 8.3.2. Survival in cold storage: Fig. 8.2.1).

The per cent emergence from mummies collected on Dennebos (Fig. 8.3.5), during July showed the same trend as those collected on Oak Valley (Fig. 8.3.2), except that more adults emerged from material collected on Dennebos. Nearly 50% of the mummies collected from Dennebos contained non-diapausing parasitoids as they emerged in less than 8 days after being removed from cold storage (Fig. 8.3.5A and B). After 8 weeks in cold storage diapause was completed by most of the parasitoids as they emerged between 8 and 11 days (Fig. 8.3.5C). In addition, overall mortality was low. The

Table 8.3.1. Percentage of each *Aphelinus mali* life stage alive and dead in mummified *Eriosoma lanigerum* from the farms Oak Valley and Dennebos.

Collection Date	FARM	LIFESTAGE ALIVE				LIFESTAGE DEAD			
		larvae	pupae	Pre-imaginal	adult	larvae	pupae	pre-imaginal	adult
16 June	**Oak Valley**	57	15	14	6	4	2	1	1
14 July	**Oak Valley**	48	32	2	4	8	2	2	2
14 July	**Dennebos**	64	30	2	0	0	2	0	2

percentage mortality in the dissected mummies (Table 8.3.1) collected from Dennebos was lower (4%) than that in mummies from Oak Valley (14%). However, on both farms the most plentiful developmental stage in the mummies was larvae followed by pupae. From the mummies collected on Dennebos 51% *A. mali* adults emerged after cold storage when placed at 25°C on 21 October 1993. The higher survival of diapausing larvae from Dennebos compared to those from Oak Valley was significant. ($Z=1.709$, $P<0.05$).

8.3.4. Postdiapause development at different temperatures

Mortality increased from the 16th of June (the day after collection when mummies were dissected) to the 21st of October, when the first replicate (at constant temperatures)

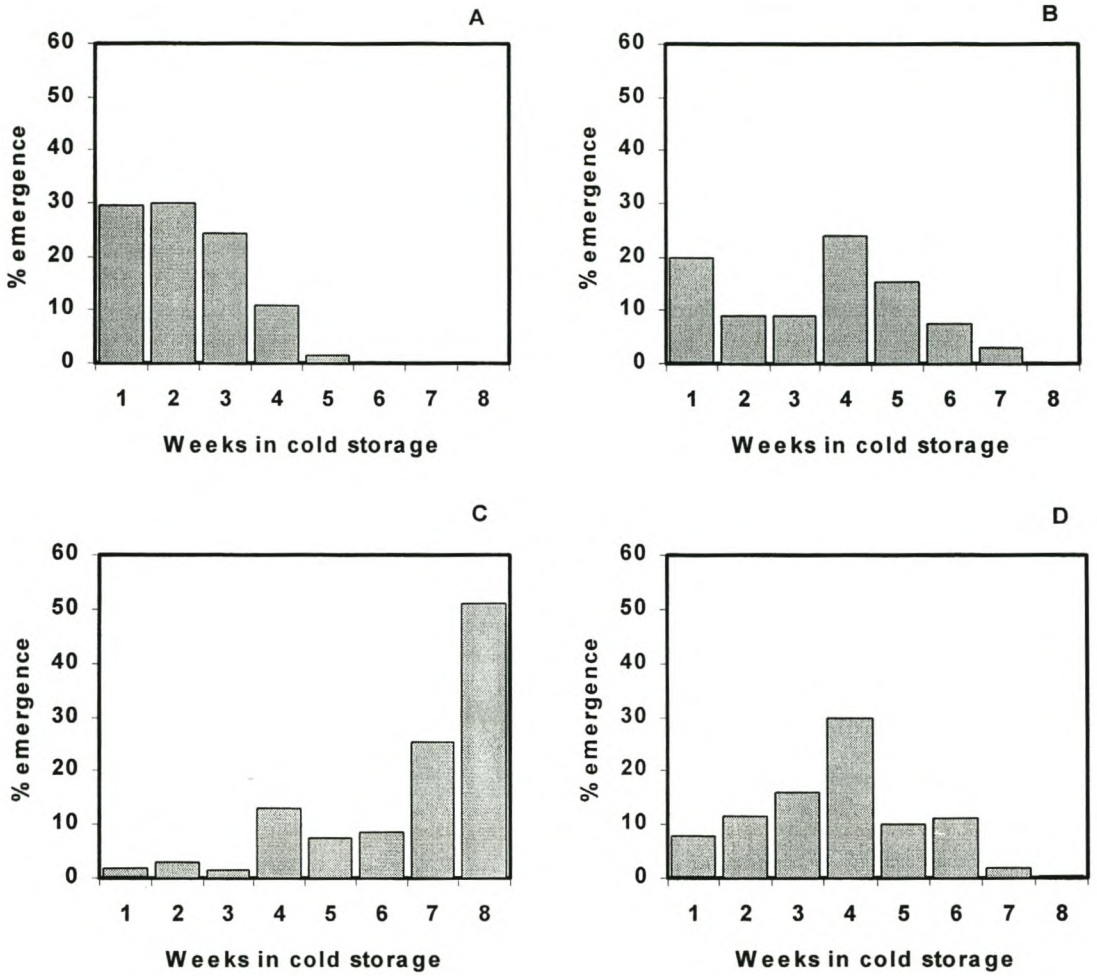

Fig. 8.3.5. Percentage of *Aphelinus mali* adults emerging after 4 days (A), between 5 and 7 days (B), between 8 and 11 days (C) and after 11 days (D), at 25°C from mummies collected on Dennebos on 14 July 1993 and kept for various periods in cold storage.

was started. Of the 200 mummies dissected on the 16th of June 1994 all stages were alive (Table 8.4.1). The mummified aphid population contained all developmental stages of *A. mali* although approximately 50% were fully grown larvae. Of the 200 mummies dissected before the experiment was started, 69% were alive. The remaining were dead. Most of these were larvae. This indicated again that even though *A. mali* overwintered as a fully grown larva not all the larvae were in diapause or postdiapause.

Table 8.4.1. The percentage for each life stage of *Aphelinus mali* present inside parasitised *Eriosoma lanigerum* mummies collected on the 15th of June 1994 from Oak Valley when they were dissected on the 16th of June and 21st of October 1994 after the mummies were kept in cold storage.

	16 June 1994	**21 October 1994**	
LIFE STAGE	**% ALIVE**	**% ALIVE**	**% DEAD**
LARVAE	54	40	13.5
PUPAE	29.5	24	7
HALF MATURE	13	5	4.5
ADULTS	3.5	0	6

Temperature affected the rate of postdiapause development of *A. mali* (Table 8.4.2). There was no difference ($F_{3,11}$=0.187; P=0.902) between the percentage

emergence at the different temperatures. The percentage emergence from the 600 mummies observed at each temperature was 19.5% at 15°C; 20.3% at 20°C; 19.9% at 25°C and 21.2% at 27°C. Between 52.5 and 60.7% ($\bar{x}$=57.2) of the emerged parasitoids were males.

Mean (±SD) times to emergence in the three replicates ranged from 24.25±1.565 days at 15°C to 7.43±0.076 days at 27°C for males. Those for females ranged from 25.28±2.076 days at 15°C to 7.70±0.126 days at 27°C. Development times were computed using pooled data from the three replicates (Table 8.4.2).

There were no significant differences in the linear relationships either between male and female postdiapause developmental rates (1/days to emergence) and constant temperatures or between the three replicates ($F_{(10,12)}$=0.7593; P= 0.336). The minimum developmental temperature (t_o) for postdiapause *A. mali* was 10.15°C (Fig. 8.4.1).

Table 8.4.2. Mean (±SD) postdiapause developmental time of *Aphelinus mali* at 4 constant temperatures.

TEMPERATURE °C	N	DEVELOPMENTAL TIME (days)
15	117	24.96(±1.96)
20	121	14.04(±1.27)
25	119	8.48 (±0.61)
27	127	7.54 (±0.85)

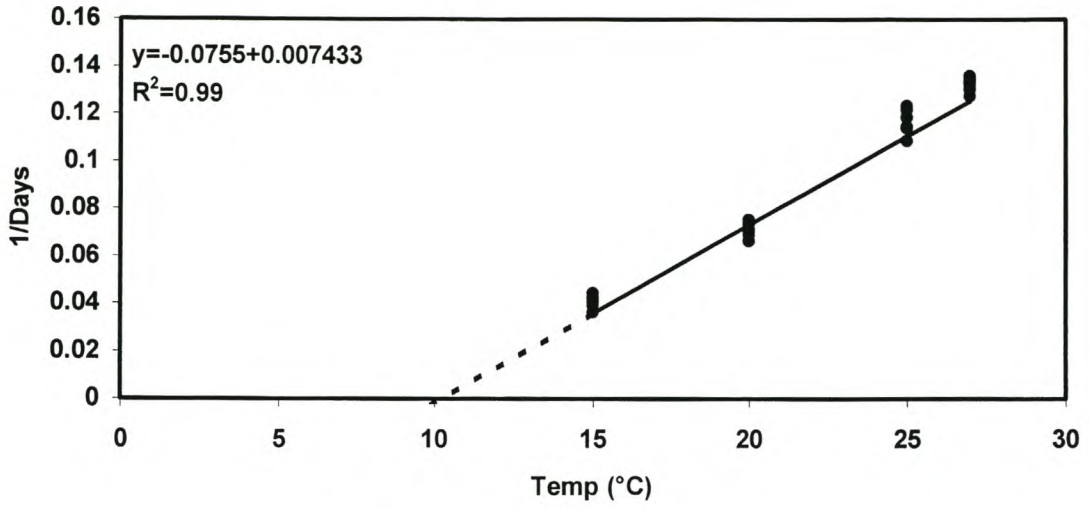

Fig. 8.4.1. The linear relationship between *Aphelinus mali* postdiapause developmental rate (% total development /day) and constant temperature.

8.4. Discussion

High constant temperatures (above 27°C) did not adversely affect *A. mali* (Table 8.1.1 and 8.1.2) as was the case with *E. lanigerum* (Chapter 3), indicating that there is differential tolerance to high temperatures between the pest and the parasitoid.

Half of the mummified aphid population in the orchard during early winter contained *A. mali* individuals that were not in diapause as they emerged within 7 days after they were placed in growth promoting conditions (25°C). *E. lanigerum* overwinters on the tree (Chapter 4) and can therefore, provide hosts for the parasitoids that emerged during this time. *A. mali* adults were found on yellow traps in the orchard during the winter indicating that temperatures were sufficiently high for their development (Chapter

4) in South African orchards. This is contrary to the results of Trimble *et al.* (1990) who found that most parasitoids entered diapause during winter in the Netherlands.

Diapause was not terminated when diapausing larvae were placed at growth promoting conditions (25°C). Tauber & Tauber (1976) found that in many insects no specific stimulus actively terminated diapause.

Non-diapausing *A. mali* (pupae or adults) inside mummified aphids tolerated low temperatures (1-4°C) for up to 8 weeks and the developing larvae probably for 10-12 weeks. The postdiapause larvae also tolerated these low temperature for long periods. This was explained by Trimble *et al.* (1990) who found that postdiapause individuals retained many of the physiological characteristics of the diapause phase, such as low temperature tolerance (Tauber & Tauber 1976).

The theoretical threshold temperature for development (t_o) of 10.15 °C for postdiapause larvae was near the t_o of 10.27°C of non-diapausing *A. mali* larvae reared in the laboratory. This value was higher than that of 9.4°C found in Washington (Walker *et al.* 1988) and at De Bilt in the Netherlands (Trimble *et al.* 1990). In France, Bonnemaison (1965) found that postdiapause *A. mali* emerged from mummies at 8.5 and 9.5°C. Geographical variation in threshold and developmental time has been demonstrated for other insects, such as the cabbage aphid and its parasitiod in Australia, U.K. Canada and the United States (Campbell *et al.* 1974).

The minimum threshold temperature for postdiapause *A. mali* was much higher (10.15°C) than that of its host, *E. lanigerum* of 4.48°C (Chapter 3). This was also the case in other countries (Bodenheimer 1947, Bonnemaison 1965, Walker *et al.* 1988, Asante & Danthanarayana 1992). This means that the spring temperatures which are favourable for

E. lanigerum development will be too low for mass development of *A. mali* and the aphids would escape biological control (Walker *et al.* 1988, Asante & Danthanarayana 1992). Predators and parasitoids of insects generally tend to have higher activity and developmental thresholds than their hosts (Campbell *et al.* 1974). By developing slower than the host the parasitoid ensures the continued availability of hosts and thus its own survival (De Bach 1964).

As most of the parasitoids appeared at the end of August when postdiapause was completed the chemical sprays applied to combat delayed foliation, *Q. perniciosus* and *Pseudococcus* pests (Nel *et al.* 1999) removed most of them (Chapter 4). Therefore, their numbers were too low for biological control of the *E. lanigerum* crawlers that moved up from the roots into the apple trees in large numbers during spring (Chapter 4).

Biological control is most likely to be successful when the *E. lanigerum* population is distributed in numerous small colonies and few large ones (Mueller *et al.* 1992). This implies that biological control must begin early in the season when the average size of colonies is small and that parasitism must persist unabated to restrict colony growth (Mueller *et al.* 1992). Twigs with diapausing *A. mali* kept in cold storage until after the upward migration of *E. lanigerum* crawlers, could then be released to augment the number of parasitoids parasitising aphids during early summer. These parasitoids will also escape the negative effect of chemicals sprayed at the end of winter, as they will be placed in the orchards after the sprays have been applied.

Based on the above results it is recommended that twigs with mummified aphids, that are usually pruned off at the end of winter, are removed and stored at temperatures under 5°C from June to July. Survival for at least to 17 weeks in cold storage of *A. mali*

in these mummies was good (30%). This period of survival would be sufficient for placing mummies on prunings into the orchards during the next spring to achieve early season parasitism of *E. lanigerum* colonies. Mummies should be kept in cold storage for at least 12 weeks if collected during June to ensure that diapause development has been completed.

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CHAPTER 9

COLONY COMPOSITION OF *ERIOSOMA LANIGERUM* (HAUSMANN) AND WITHIN COLONY PARASITISM BY *APHELINUS MALI* (HALDEMANN).

9.1. Introduction

Childs and Gillespie (1992) described *Aphelinus mali* (Hald.) as a model parasitoid. Its freedom from hyperparasitoids, short life cycle, unique host selection capability, ability to survive for an almost indefinite period in cold storage, immunity to orchard sprays, etc., makes its presence highly desirable in any orchard where *Eriosoma lanigerum* (Hausmann) is a pest.

However, *A. mali* is ineffective as a biological control agent under cooler climatic conditions (Miller 1947, van Lenteren 1990, Asante & Danthanarayana 1992). In areas where *A. mali* has not provided complete control of *E. lanigerum* a differential response to temperature has been postulated as the factor reducing control. This would cause a lag between aphid population development and parasitoid activity (Bodenheimer 1947, Evenhuis 1958, Walker *et al.* 1988, Chapter 8).

In South Africa a high percentage parasitism of *E. lanigerum* colonies has often been accompanied by an increase in the number of colonies (Chapter 4). This suggests that the degree of parasitism within colonies is not sufficient for control.

The present study was designed to investigate the age composition of *E. lanigerum* colonies, as this can influence the effectiveness of biological control, as well as to determine the percentage parasitism within each colony.

9.2. Material and methods

Once a month for four seasons (January – August 1996 to 1999) ten infested branches were randomly collected on Molteno in the Elgin district and brought to the laboratory in cool bags. One colony was removed from each branch. Early in the season only a few branches had colonies. Therefore, it was sometimes necessary to use more than one colony from a single branch. As soon as the colonies became visible and were taken to the laboratory for dissection all the developmental stages of *E. lanigerum* were recorded. The aphids from each colony were sorted into the different instars (Chapter 2) and counted. They were dissected individually to determine if they were parasitised by *A. mali*. The number of parasitoid eggs or larvae in each individual was noted as it is known that more than one egg may be deposited in the same aphid (Lundie 1924). The number of mummified aphids was also counted as well as the number of empty aphid shells. This gave an indication of the stage of parasitism. Due to the effect of environmental factors (e.g. temperature, relative humidity, host plant condition, suitable feeding sites, host density etc.) on host size, some mummified aphid nymphs can be larger than mummified

adults (Asante & Danthanarayana 1993). Therefore the mummies were not categorised as nymphs or adults.

The relationship between the number of colonies in the orchard and the number of individuals within each colony was investigated by regressing log average number of colonies per tree on log average individuals per colony. The relationship between parasitised colonies in the trees and parasitised individuals within colonies was investigated by regressing the working logit (Cox 1970) parasitised colonies per tree on the working logit parasitised individuals in colonies.

The time at which diapause started in the orchard during 1999 was determined by placing 50 mummies, which were randomly removed from twigs, in a petri dish at room temperature. This was done once a month from January until June 1999. The mummies were examined after 10 days for emergence of the parasitoids. The mummies from which parasitoids had not emerged were dissected to determine whether or not the parasitoids were alive. If the dissected mummies contained live final instar larval parasitoids they were considered to be in diapause as non-diapause parasitoids emerge within 7 days (Chapter 8).

9.3. Results and discussion

During summer and autumn the first three developmental stages made up more than 50% of the individuals in the colonies (Fig. 9.1). At the end of winter during most seasons the third and fourth instars were prevalent (Fig. 9.1). During the 1998 season no live adult aphids were recorded during August and only a few during August 1999. This may have been because at this time of the year most of the aphids were parasitised and

the adults were mummified. Although the duration of the adult stage was longer than that of the other stages of *E. lanigerum* (Chapter 3) the proportion of adults within the colonies was lower than that of the other developmental stages for most of the season. When fewer adult aphids were present the production of first instar nymphs declined. This was the case during the 1997 season when only 2 adults were found on the second last date (Fig. 9.1). On the following date only a small percentage of the colonies contained first instar nymphs. This was also apparent at the end of the 1998 season (Fig. 9.1).

There was a weak correlation between the log average number of individuals per colony and the log average number of colonies per tree (R^2=0.1999; P=0.01) (Fig. 9.2). During the first season the largest colonies were found while the least number of parasitised aphids within colonies was recorded (Fig. 9.3). *E. lanigerum* counts in the aerial parts of the trees were higher during this season than during any of the others (Chapter 4, Fig. 4.2.3.), and levels of parasitism were lower than during any other. When the number of parasitised aphids increased the number of live individuals in each colony decreased (Fig. 9.3). Thereafter the number of parasitised aphids also decreased.

The winged form of *E. lanigerum* was mostly found from February until April, except during 1996 when colony numbers were still high late in the season (Fig. 4.2.3, Chapter 4) and winged aphids were still recorded in May 1996 (Table 9.1). Usually immature stages of the winged form were recorded as most of the adults flew away or dropped from the branches when they were cut from the apple tree. The sexual form was never recorded in any of the *E. lanigerum* colonies collected from the orchard. However,

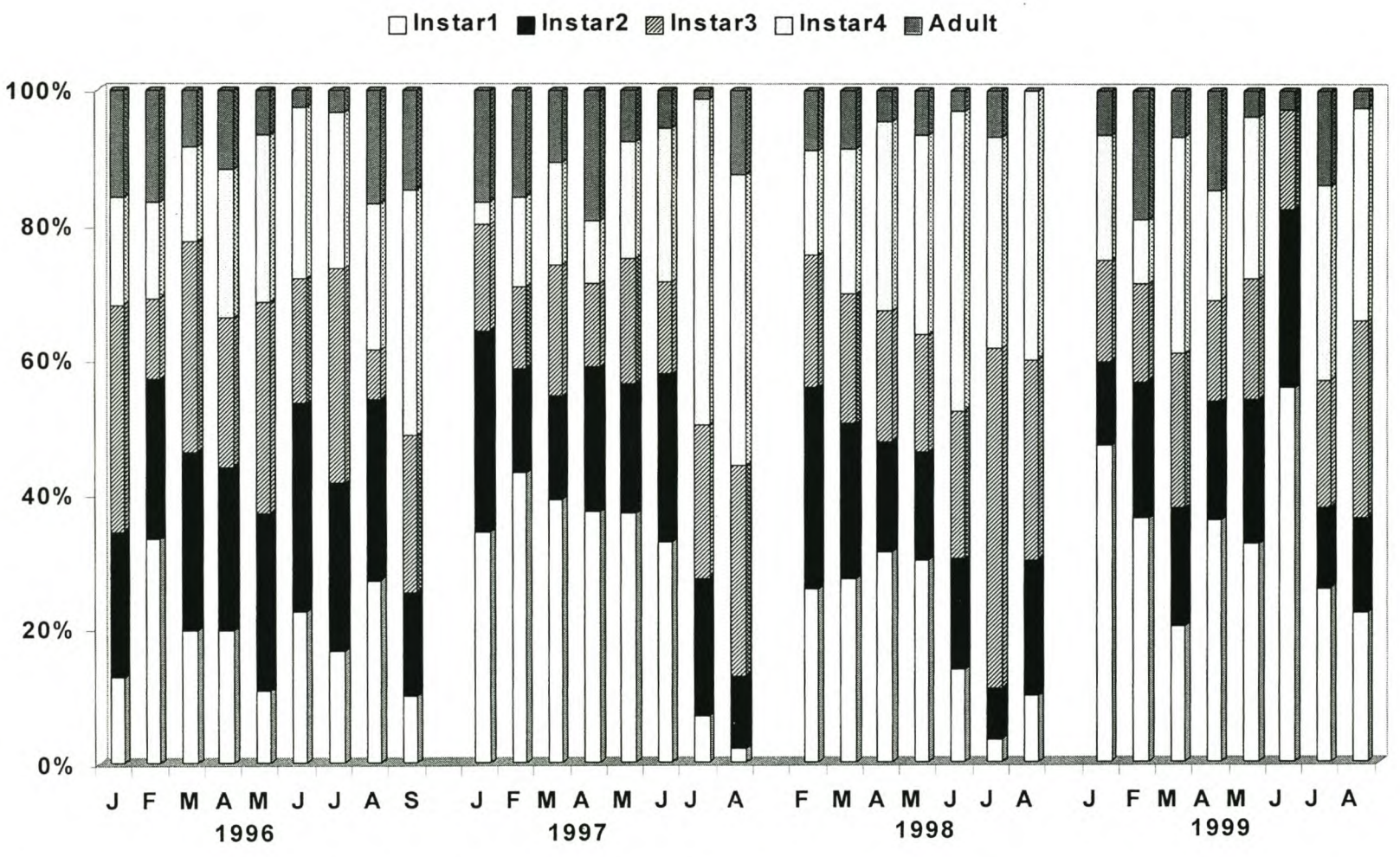

Fig. 9.1. Average percentage of the five developmental stages of *Eriosoma lanigerum* per colony for four seasons.

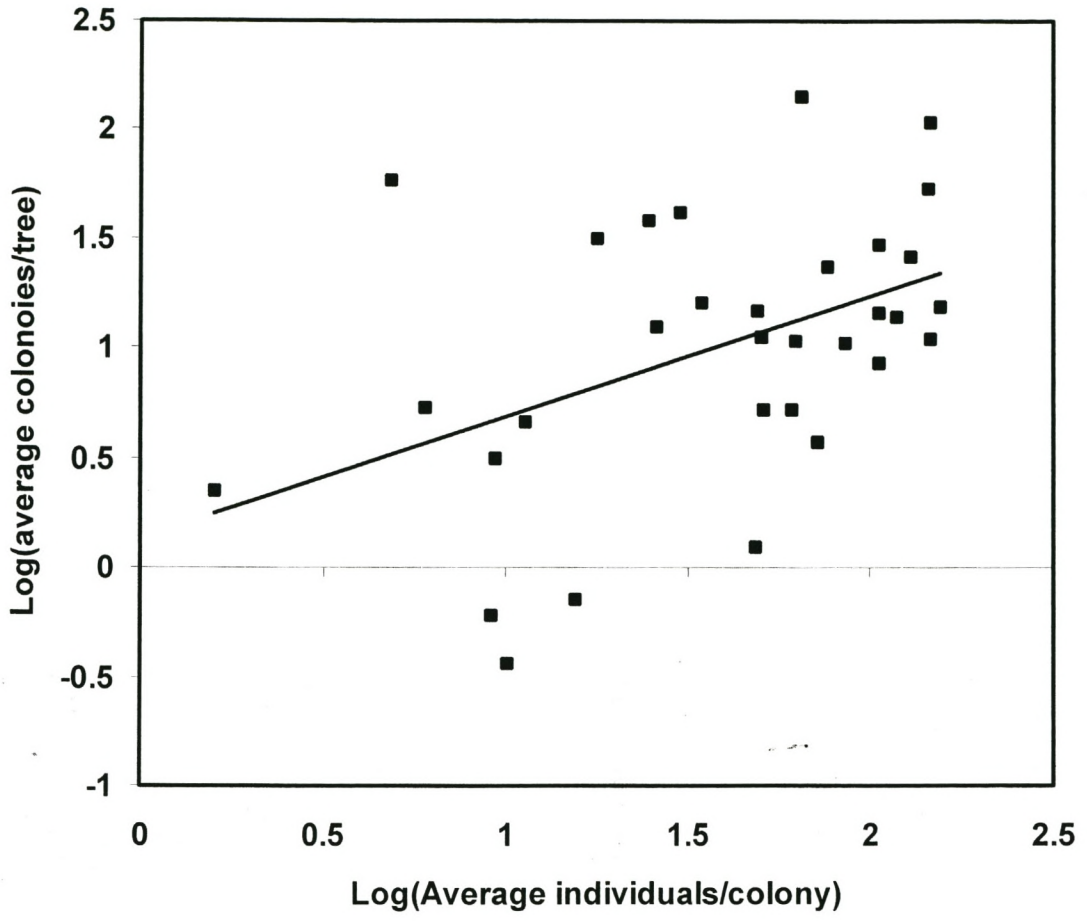

Fig. 9.2. Relationship between log (average number of *Eriosoma lanigerum* colonies per tree) and log (average number of individuals per colony). Y = 0.1362 + 0.5485(x).

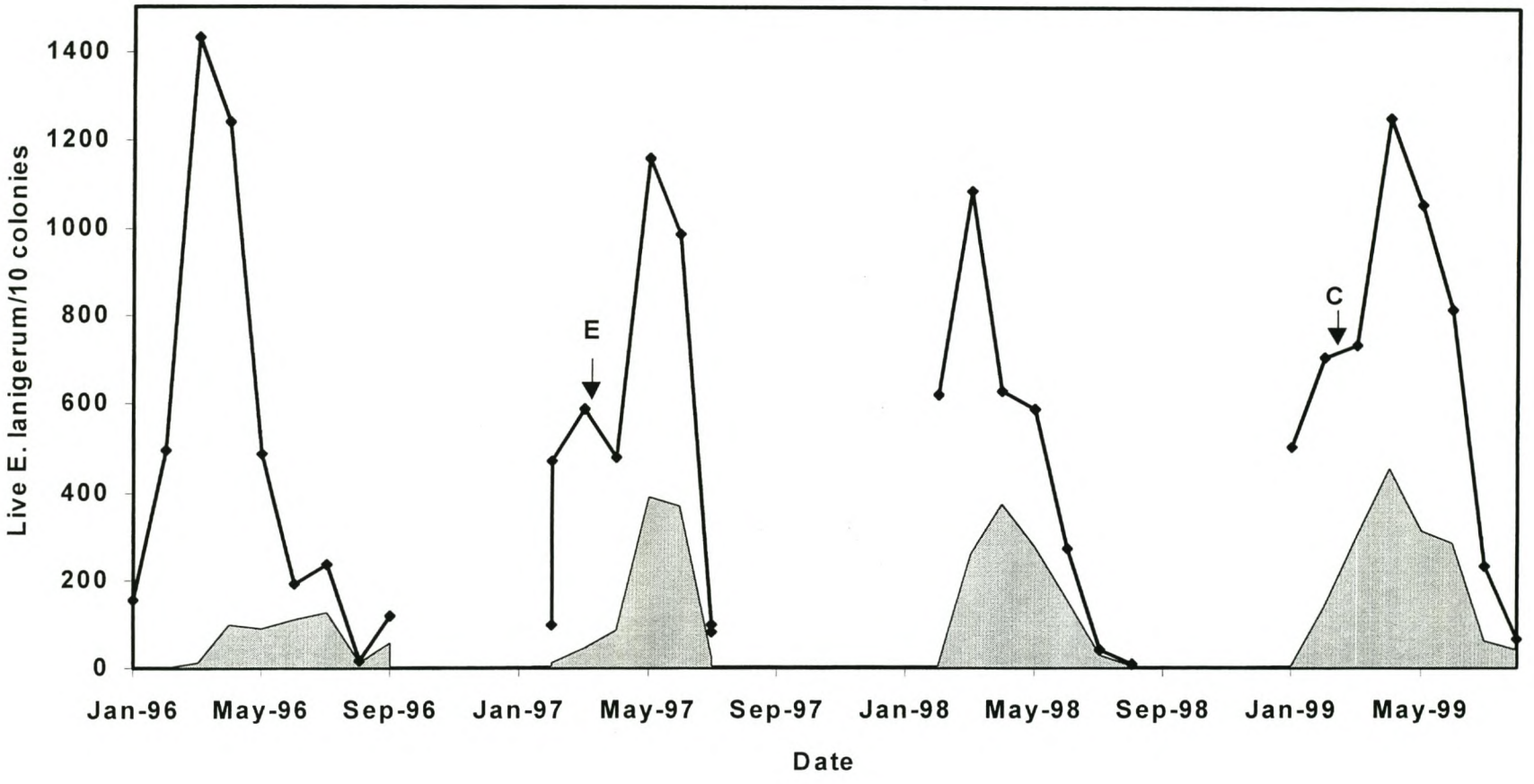

Fig. 9.3. The total number of live *Eriosoma lanigerum* in 10 colonies (line) and the number of *E. lanigerum* containing *Aphelinus mali* larvae or eggs (shaded area) with the times at which endosulfan (E) and chlorpyrifos (C) were applied.

the sexual form was obtained from winged adults placed in a petri dish on damp filter paper. This indicated that the winged adults were the autumn migrants which were supposed to give rise to the sexual males and females that lay the winter egg of *E. lanigerum* (Baker 1915, Becker 1918, Crane *et al.* 1936). This winter egg was never recorded in any of the colonies examined. Larvae and eggs of *A. mali* were also found inside the winged form of *E. lanigerum* (Table 9.1) and some of the mummified aphids were also immature or adult winged aphids. The appearance of winged aphids together with parasitism by *A. mali* were followed by a drop in the number of live aphids in each colony (Fig. 9.3). In Australia there was density dependant regulation of *E. lanigerum* by means other than parasitism. The production of large numbers of winged females at peak population densities in February and March resulted in a decline in colony numbers (Asante *et al.* 1993). Hely *et al.* (1982) and Thwaite & Bower (1983) also found that the appearance of winged females caused a reduction in the population.

Parasitised aphids were found from the end of January in each season until aphids were no longer visible on the trees at the end of winter. All the developmental stages of *E. lanigerum* were parasitised by *A. mali* (Fig. 9.4). However, the third, fourth and adult stages were preferred. This was also the case in Australia (Asante & Danthanarayana 1993). Mummified aphids were not included in estimates of the percentage of each instar that was parasitised. Therefore, the actual percentage parasitism of the later developmental stages was probably higher than is shown in Fig. 9.4. The percentage of parasitised live aphids reached a peak at the end of winter of 1996 and 1998 when nearly 70% parasitism occurred within the colonies (Fig. 9.4). The total percentage parasitism including mummies still containing parasitoids reached 90% (Fig. 9.4) during the 1996

Table 9.1. Percentage winged *Eriosoma lanigerum* in colonies collected in the orchard from the 1996 to the 1999 season with the percentage of winged aphids that were parasitised by *Aphelinus mali*.

Date	% Winged aphids in colonies			% Winged aphids Parasitised		
	Third instar	Fourth instar	Fifth instar	Third instar	Fourth instar	Fifth instar
96/03/06	1.82	1.82	0	0	0	0
96/04/03	2.5	5	0.48	12.9	19.35	33.33
96/05/02	0.41	0	0	50	0	0
97/02/19	0.211	0	0	0	0	0
97/03/05	9.81	3.55	0	8.62	4.72	0
97/04/02	0.628	1.05	0	0	40	0
98/03/04	1.38	1.29	0	20	64.29	0
99/02/17	0.423	0	0	0	0	0
99/03/17	7.08	8.31	0.14	42.3	50.82	0
99/04/15	0.24	0.24	0	33.33	33.33	0

season and more than 80% during the 1998 season (Fig. 9.4). Many of the earlier instars escaped parasitism as they were under other aphids and mummified aphids. Live first instar crawlers were also found inside the empty mummified aphid bodies during winter. First and second instar aphids were parasitised when parasitoid activity was high and insufficient numbers of the preferred older aphids were available.

Superparasitism was recorded during all four seasons (Table 9.2) and up to 9 larvae and eggs were found inside one aphid. More than five larvae were only recorded in fourth instar aphids. As the number of parasitoids increased (Chapter 4, Fig.4.4.2 and

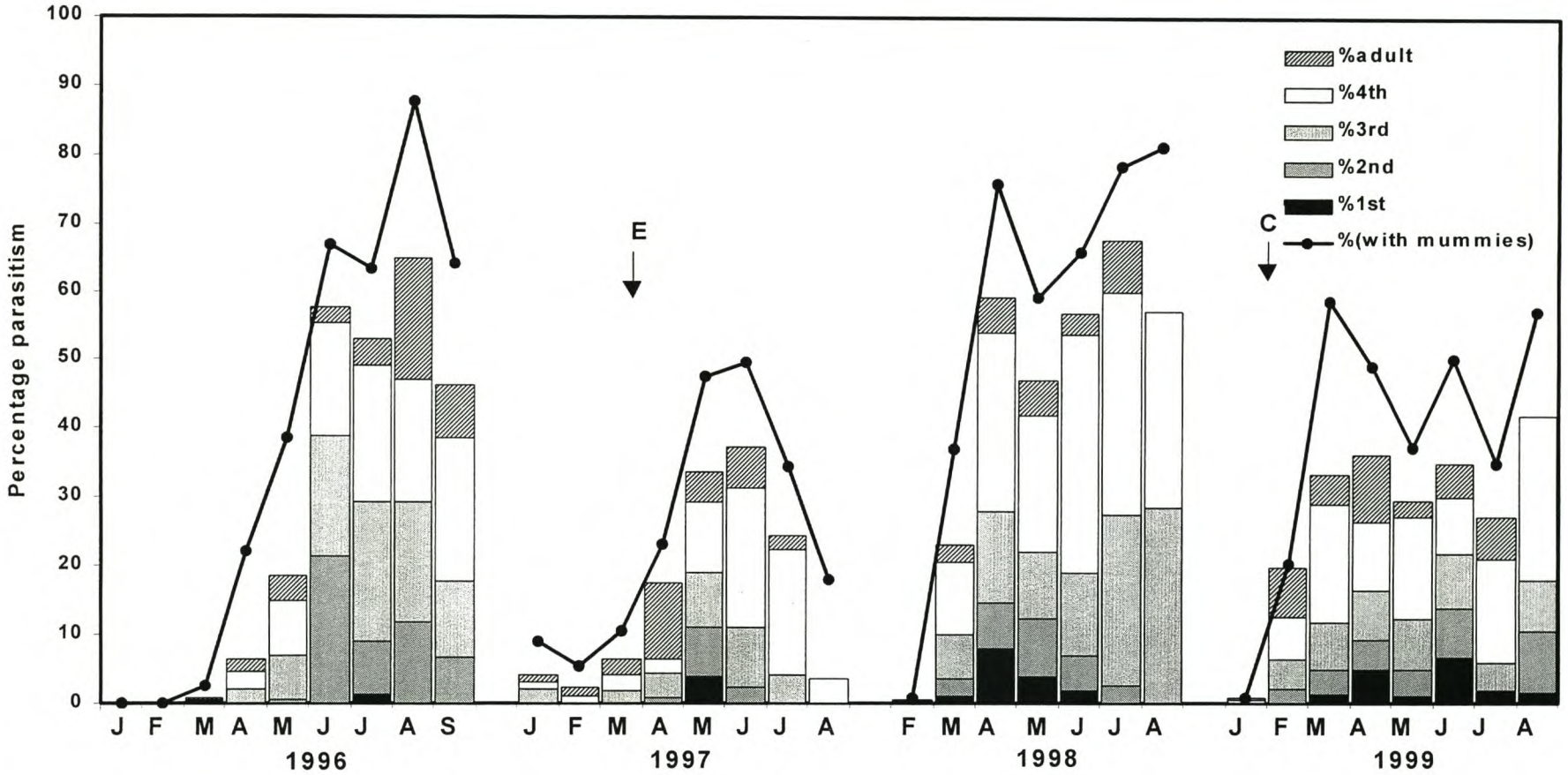

Fig. 9.4. Percentage of each developmental stage of *Eriosoma lanigerum* parasitised, excluding the mummified aphids (bars), and the total percentage parasitism including unemerged mummies (line) with the times at which endosulfan (E) and chlorpyrifos (C) were applied.

4.4.3), fewer aphids were available for parasitism. Therefore, superparasitism was more prevalent during these times of the season (Table 9.2.). During the 1998 season high temperatures were recorded (Table 4.2 in Chapter 4) which were more favourable for the parasitoid than the aphids (see Chapter 3 and 9, Walker *et al.* 1988, Asante & Danthanarayana 1992). During this season the highest number of parasitoids (9) were found in a single aphid (Table 9.2). When 20% of the live aphids were parasitised (Fig. 9.4) superparasitism was recorded (Table 9.2). Although the colonies were large not all the aphids were available for parasitism. Large *E. lanigerum* colonies often consist of a dense mass of individuals with adults and older nymphs on the surface and the smaller nymphs underneath (Asante & Danthanarayana 1993).

Parasitisation of the earlier instars will have a stronger depressing effect on the host population than parasitisation of the fourth instar nymphs and adults (Mueller *et al.* 1992). If the third and subsequent *E. lanigerum* instars are parasitised they still develop to the adult stage and produce nymphs before mummification (Asante & Danthanarayana 1993).

There was a good correlation (R^2=0.606, P<0.001) between the working logit average number of parasitised *E. lanigerum* colonies counted in the trees and the working logit of the average number of individuals parasitised within colonies (Fig. 9.5). Early in the season parasitism was low in both the parasitised colonies in the trees and the number of individuals parasitised in each colony and both reached high numbers at approximately the same time during the season. However, there were more visible fluctuations in parasitism within colonies (Fig. 9.4) than of the colonies counted in the orchard (Chapter 4) as a colony was regarded as parasitised when at least one aphid was mummified.

Table 9.2. The number of *Eriosoma lanigerum* dissected, the number parasitised by *Aphelinus mali* with the number of parasitoid larvae found in the aphids.

Date	Aphids Dissected	Aphids parasitised	*A. mali* larvae/eggs inside each aphid									Total
			1	2	3	4	5	6	7	8	9	
96/6/20	192	111	93	12	3	2	1	0	0	0	0	139
96/7/7	236	125	125	0	0	0	0	0	0	0	0	125
96/8/6	17	11	10	1	0	0	0	0	0	0	0	12
96/9/4	119	55	54	1	0	0	0	0	0	0	0	56
97/5/5	1154	385	349	28	7	1	0	0	0	0	0	430
97/6/4	620	379	350	22	6	1	0	0	0	0	0	416
98/3/4	1077	257	203	40	11	2	0	0	0	0	1	333
98/4/15	630	372	258	81	25	7	0	1	0	0	0	529
98/5/13	589	277	211	47	10	4	2	2	0	0	1	344
98/6/10	273	155	136	12	5	1	0	0	0	1	0	187
98/15/7	40	27	21	6	0	0	0	0	0	0	0	33
99/2/17	709	140	128	11	1	0	0	0	0	0	0	153
99/3/17	736	302	263	33	4	2	0	0	0	0	0	349
99/4/15	1252	457	395	52	10	0	0	0	0	0	0	529
99/5/11	1057	312	269	33	8	2	0	0	0	0	0	367
99/6/7	815	286	250	32	4	0	0	0	0	0	0	322
99/7/12	232	63	61	2	0	0	0	0	0	0	0	65
99/8/10	67	28	23	5	0	0	0	0	0	0	0	33

The first diapausing larvae of *A. mali* recorded during the 1998/99 season were found during April 1999 (Table 9.3). Most of the mummies (92%) collected in the middle of March 1999 were not in diapause. During May 1999 a higher percentage of mummies (14%) contained larvae in diapause and in the first week in June 50% of the mummies contained *A. mali* in diapause (Table 9.3). This indicated that diapause started during the middle of autumn (after March, but before June). It was found that approximately 50% of the parasitoids entered diapause in the Grabouw area (Chapter 8) which corresponds well with these results.

Table 9.3. Percentage of mummified *Eriosoma lanigerum* with non-diapausing parasitoids (emerging within 10 days), those containing dead parasitoids and parasitoids in diapause.

Date collected	% Non – diapausing	% Dead	% in Diapause
99/3/17	92	8	0
99/4/14	90	8	2
99/5/11	70	16	14
99/6/7	44	6	50

Two sprays were applied for the control of *E. lanigerum* during the period of investigation in this orchard. An endosulfan spray applied during March 1997 caused a temporary decline in the number of aphids per colony (Fig. 9.3). Chlorpyrifos was sprayed during January 1999. Some of the colonies were visibly influenced by the

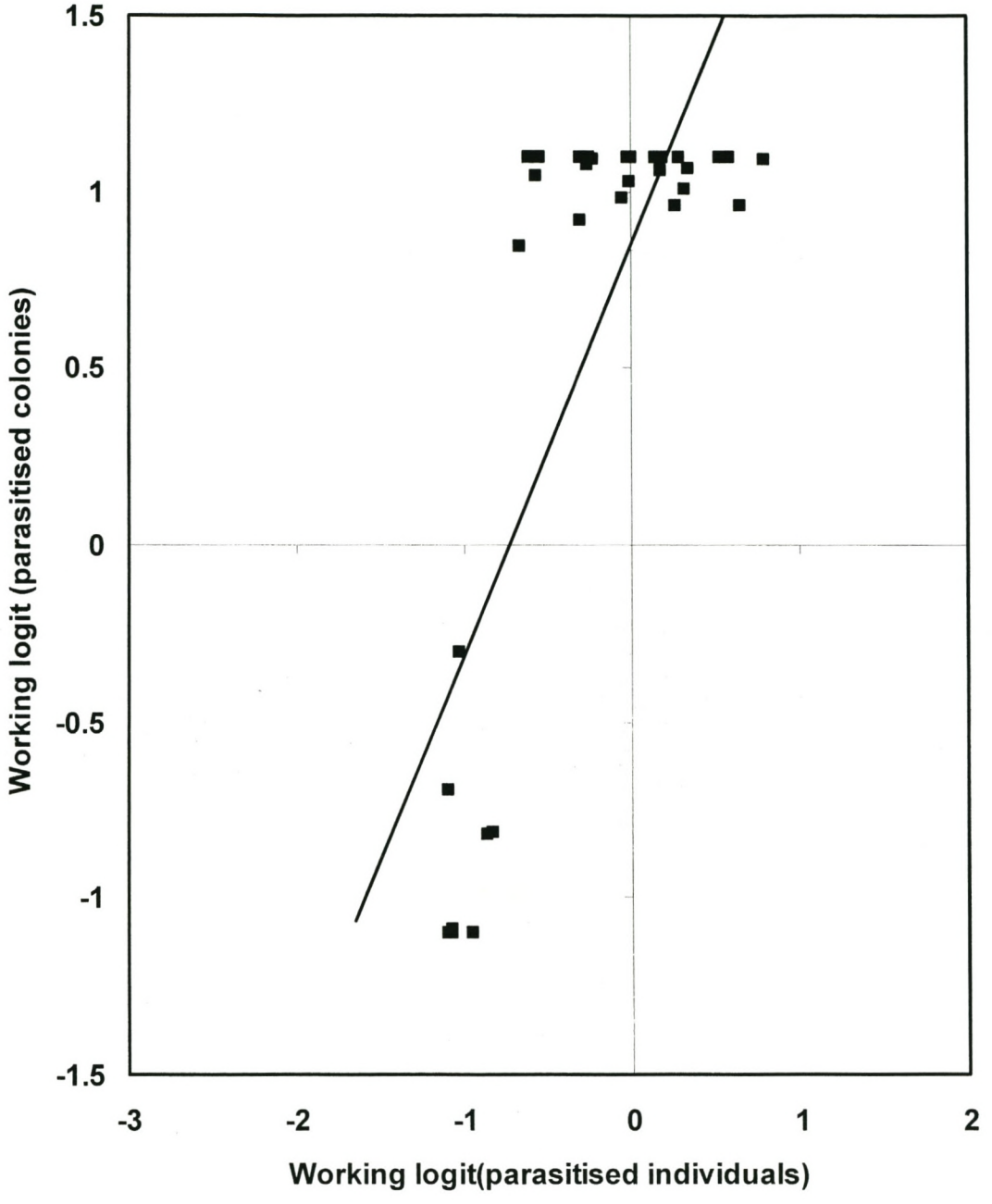

Fig. 9.5. Relationship between working logit (average parasitised colonies per half tree) and working logit (average parasitised individuals per colony).

$Y = 0.8468 + 1.62(x)$.

chemical and contained many aphids without the typical waxy "wool", while others contained only healthy aphids subsequent to the spray application. A few colonies (n = 3) collected from the inside of the apple trees were not influenced by the chemical and contained a large number of individuals (average = 221.33 per colony) while those that were sprayed (n = 7) had only a few individuals (average = 6.14 per colony). Therefore, the chlorpyrifos had a dramatic influence only on those colonies contacted by the spray. This also illustrated one of the problems associated with chemical control of *E. lanigerum*. Colonies protected from sprays by, for example fruit clusters, can cause rapid re-infestation.

The endosulfan spray applied in 1997 did not have a negative effect on the parasitoid as the percentage of *E. lanigerum* parasitised by *A. mali* steadily increased after the spray. However, the chlorpyrifos spray applied in the 1999 season probably had a negative effect on the parasitoid as the percentage parasitism decreased after the spray was applied (Fig. 9.4).

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CHAPTER 10

THE SUSCEPTIBILITY OF *APHELINUS MALI* (HALDEMAN), THE NATURAL ENEMY OF *ERIOSOMA LANIGERUM* (HAUSMANN), TO PESTICIDES USED IN APPLE ORCHARDS.

10.1. Introduction

The use of pesticides against harmful insects and mites frequently disrupts biological control. Most natural enemies, on which biological control of pest species depends, are highly sensitive to these agricultural chemicals. In addition, the development of resistance is common among many pest species, but is rare among their natural enemies (Croft 1982). Lack of pesticide resistance among beneficial predators and parasitoids is a serious constraint in increasing their role and efficacy in integrated pest management (IPM) programs. Conservation of natural enemies by manipulating or changing spray programs to include selective pesticides, which are not always as effective as the non-selective alternatives, may reduce the level of control of key pest populations. The result is that early-season pesticide applications frequently endanger the survival of biological control agents, committing the crop to chemical control for the remainder of the season (Havron *et al.* 1987).

Implicit in IPM is the maximum utilisation of natural enemies, supplemented by the selective use of insecticides when necessary (Hsieh & Allen 1986). The importance of integrating chemical and biological control has become increasingly apparent since the advent of the use of synthetic organic pesticides. Use of pesticides incompatible with parasitoid and predator activity has produced target pest resurgence and secondary pest outbreaks throughout the world's agroecosystems (Luck *et al.* 1977, Metcalf 1986). These ecological disruptions have resulted in increased crop damage, increased need for additional pesticide applications, accelerated evolution of pesticide resistance, and increased general contamination of the environment (Rosenheim & Hoy 1988).

Outbreaks of *Eriosoma lanigerum* (Hausmann) have also often occurred as a result of pesticide applications, which have detrimental effects on biological control (Penman & Chapman 1980, Cohen *et al.* 1996). It was therefore decided to determine the effects of different pesticides used in South African apple orchards on the parasitoid, *Aphelinus mali* (Hald.), under controlled conditions.

10.2. Material and methods

10.2.1. Adults

(i) Preparation of test parasitoids

Twigs with parasitised woolly apple aphid mummies were collected from the apple orchards in the Elgin district. All the live parasitoids were removed from the twigs after which the twigs were placed in an incubator at 25°C. After 24 hours the newly emerged adults were removed using an aspirator and kept inside small glass tubes

plugged with cotton wool dipped in a sugar-water solution, until needed. The females were then separated from the males.

(ii) Exposure cage

Exposure of the parasitoids was carried out in cages similar to those described by Kühner *et al.* (1985). The cages consisted of an aluminium frame 2cm high with internal measurements of 10 x 10 cm, sandwiched between two glass plates 3 mm thick (Fig. 10.1). The glass plates were kept in place with sticky tape. Three holes (7 mm diameter) were made in each of two sides of the frame. Five holes were covered with fine gauze and one was left open through which the wasps were supplied with a sugar-water solution in cotton wool. One of the centre holes had a small pipe extension 2 cm long (5 mm diameter).

(iii) Procedure

Pesticides were applied at field concentration using a Potter's spray tower (Potter 1952). A volume of 400µl was applied to both of the glass plates. This delivered 0.1-0.26 ml/cm^2. Water treated plates were used as control. After deposits were dry the cages were assembled and 20 - 50 females placed inside each cage. The parasitoids were placed in a cool room for a short period (approximately 5 minutes) to immobilise them before they were placed in the cages. Six such cages were ventilated using an aquarium pump. The air flow was measured by passing it through a flowmeter. The air in the cells was replaced every 25 seconds. In addition the humidity of the air was regulated at 80% RH

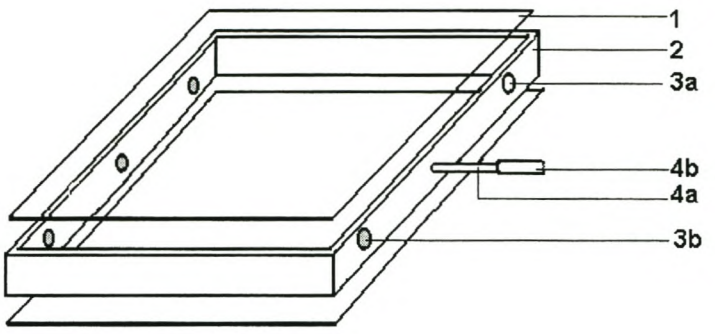

Fig. 10.1. Aluminium test cage. 1 = glass plate, 2 = aluminium frame, 3a = hole plugged with cotton wool dipped in sugar water, 3b = ventilation hole with gause, 4a = aluminium pipe extension, 4b = plastic tube from aquarium pump.

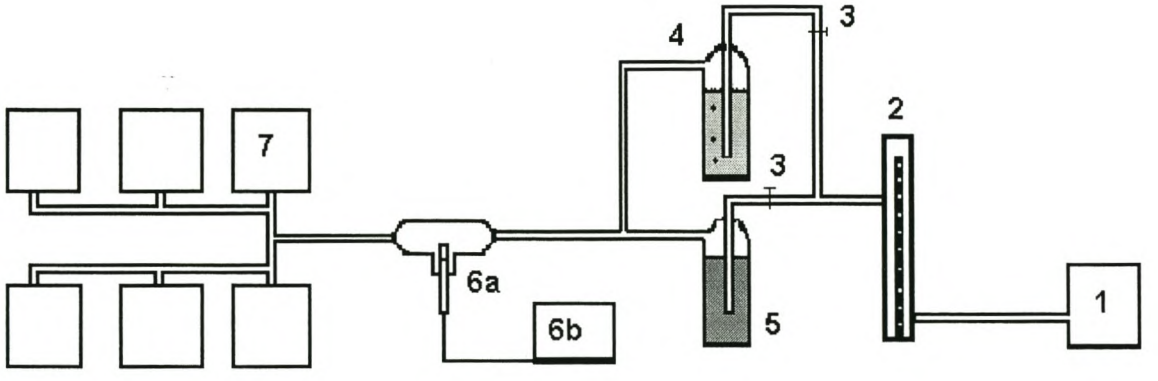

Fig. 10.2. Assembled apparatus for testing the effect of chemicals on *A. mali*. 1 = Aquarium pump, 2 = Airflow meter, 3 = clamp, 4 = flask with water, 5 = flask with silica gel crystals, 6a = Probe for measuring temperature and humidity, 6b = data logger, 7 = aluminium cage.

by splitting the air stream into two and passing one stream through water and the other through silica gel crystals. Laboratory clamps were used to adjust the air flow in the two streams to achieve the desired relative humidity (80%). The two air streams were then brought together and passed through a glass chamber containing a temperature and humidity probe connected to a data logger. A glass manifold was then used to split the air stream and pass it through the six cells (Fig. 10.2). The cages were kept inside an incubator at 25°C and a light/dark cycle of 12/12. Mortality was determined after 24, 48, and 72 hours by counting dead adults inside each cage.

10.2.2. Chemicals

The chemicals tested for their effects on *A. mali* were applied at field dose. These chemicals with their trade names and field concentrations are given in Table 10.1. Mortality counts were corrected for control mortality using Abbott's (1925) formula.

10.2.3. Fecundity

Insect growth regulators sometimes adversely affect the fecundity of adults. Therefore, the fecundity of *A. mali* was tested using surviving wasps from the experiments described above. Surviving adult females were removed after 48 hours. They were placed individually into a plastic container covering a small apple tree (10 – 20 cm high) infested with woolly apple aphid colonies. Each wasp was allowed to parasitise the aphids and was provided with a sugar-water solution in a small plastic cap at the base of each tree. Four such females were used for each test chemical. Females from cells sprayed with water were used as controls. The trees were kept in the cooled incubator at

25°C and after 72 hours the trees were examined and every day thereafter. The black mummified aphids were removed and counted.

10.2.4. Mummies

Twigs with parasitised woolly apple aphids were collected and kept in a cool room until needed. Mummies were removed from the infested twigs and placed on double-sided sticky tape on a microscope slide. Three such slides, each with fifteen mummies, were used per treatment. The slides were covered with paper and only the area with the mummies was left exposed. The slides were sprayed as described above. After being treated the slides with the mummies were placed into the ventilated aluminium cages described above. Slides treated with water were used as the control. The mummies were examined daily for the emergence of adult parasitoids. Mummies with unemerged parasitoids were dissected after 9 days to establish the developmental stage at which death occurred.

10.3. Results and discussion

10.3.1. Adults

All the insecticides, except the insect growth regulators, were highly toxic to adult *A. mali* within the first 24 hours after exposure (Table 10.2). Endosulfan used for the control of *E. lanigerum* was found to be less toxic to the parasitic wasp than chlorpyrifos and vamidothion, which are also used for this purpose. Therefore, endosulfan would be more suitable than chlorpyrifos or vamidothion as a corrective spray in integrated programs designed to give maximum biological control of *E. lanigerum* populations. In

Israel vamidothion was also highly toxic to adult *A. mali* (Cohen *et al.* 1996). Sheikh *et al.* (1989) found that both endosulfan and phosalone caused high mortality of *A. mali* adults and that phosalone was the safer of the two insecticides.

Azinphos-methyl, which killed all the adult wasps within the first 24 hours after exposure to residues in our experiments, was found to have a low toxicity by Croft (1982). In Israel azinphos-methyl had a moderate effect on the adult wasps (Cohen *et al.* 1996). This tolerance of *A. mali* in Israel was related to the long historical exposure of parasitoid populations to high concentrations of azinphos-methyl (Cohen *et al.* 1996). The development of partial resistance probably also occurred in the U.S.A. where large populations of *A. mali* can be found in orchards which have been heavily sprayed with azinphos-methyl for many years (Croft 1982). *A. mali* has also been exposed to azinphos-methyl for many years in South Africa. However, the concentration used locally is lower (175 ppm; Nel *et al.* 1999) than that used in Israel (400 ppm; Cohen *et al.* 1996) and the U.S.A. (400 ppm; Anon 1962 in Abivardi *et al.* 1998).

The two insect growth regulators, flufenoxuron and fenoxycarb, which are both used against codling moth, *Cydia pomonella* (L.), had minimal effects on *A. mali.*

The fungicides caused low mortality of *A. mali* within the first 48 hours after exposure to the chemical residues, but thereafter the corrected percentage mortality increased to between 50% and 75% for captab, copper oxychloride, bupirimate, kresoxim-methyl and benomyl (Table 10.2). Fenarimol produced a corrected percentage mortality of 23% after 48 hours, which increased slightly, to 32% after 72 hours. These results closely resemble those of other researchers. Cohen *et al.* (1996) found that penconazole had little effect on adult *A. mali,* as was the case in our experiments. In

addition, captab and copper oxychloride among others had little effect on adult *A. mali* at normal concentrations in Germany (Schneider 1958). Rawat *et al.* (1988) also found that captab and mancozeb, had no significant effect on the mortality of adult *A. mali.*

10.3.2. Fertility

Flufenoxuron and fenoxycarb applied to *A. mali* did not reduce the number of mummies produced by wasps. The average number of mummies produced per female treated with flufenoxuron, fenoxycarb and water were 7.5, 4.5 and 3.75 respectively, although this was lower than the average of 11 per female per day reported by Evenhuis (1958). Therefore, these insect growth regulators do not appear to have a harmful effect on *A. mali.*

10.3.3. Mummies

The corrected percent mortality of developing *A. mali* inside the mummified aphids was very low (Table 10.2) in most cases. The highest mortality within the mummies was recorded for with azinphos-methyl (14.7%) and chlorphenapyr (17.24%). In Israel azinphos-methyl and chlorpyrifos also had little or no effect on *A. mali* developing inside the mummified aphids (Cohen *et al.* 1982). However, in some cases the adults died soon after emergence. More than 60% of the adults emerging from mummies treated with chlorpyrifos died and nearly 30% of those emerging from mummies treated with carbaryl (XLR Plus) and fenthion died. Adults emerge from the aphid by cutting a hole in the body of the mummy. Therefore, they can ingest residues on the outside of the mummy. They may also come into contact with chemical residues on the sprayed surface

after emergence. Adults emerging six days after the insecticide was applied died soon after emergence, which could indicate long residual effects of these chemicals. Krespi *et al.* (1991) also found that when the aphid mummy was sprayed, mortality increased at the time of emergence, and especially at the time the parasitoid was cutting the emergence hole in the mummy.

The chemicals that caused low or no mortality within the mummified aphids will be safer for use in the apple IPM programs, as developing parasitoids within the mummy will escape the toxic effects of the chemicals ensuring survival of some. In an IPM program the use of endosulfan as a corrective spray for *E. lanigerum* control would be recommended as well as growth regulators for the control of codling moth and fungicides like penconazole, mancozeb, myclobutanol and iprodione against fungi.

Table 10.1. The chemicals used to test their effect on *Aphelinus mali* with their trade names and field doses.

Chemical (Active ingredient)	**Trade name**	**Field dose (per 100 l water)**
Azinphos-methyl (WP 350 g/kg)	Gusathion	50 gm
Vamidothion (EC 400 g/l)	Kilval	125 ml
Chlorpyrifos (EC 240 g/l)	Dursban	100 ml
Flufenoxuron (DC 100 g/l)	Cascade	25 ml
Fenoxycarb (WP 250 g/l)	Insegar	24 g
Deltamethrin (EC 25 g/l)	Decis	100 ml
Fenthion (EC 500 g/l)	Lebaycid	100 g
Phosalone (WP 60 g/l)	Zolone	100 g
Carbaryl (DP 50 g/kg)	Sevin	125 g
Methyl parathion (CS 240 g/l)	Penncap-M	70 ml
Endosulfan (SC 475 g/l)	Thioflo	100 ml
Prothiophos (EC 960 g/l)	Tokuthion	50 ml
Chlorphenapyr (SC 360 g/l)	Hunter	35 ml
Carbaryl (SC 480 g/l)	Sevin XLR Plus	100 ml
Penconazole (EW 200 g/l)	Topaz	22,5 ml
Captab (SC 500 g/l)	Kaptan Flo	200 ml
Copper oxychloride (WP 850 g/kg)	Copper oxychloride	250 g
Bupirimate (EC 250 g/l)	Nimrod	60 ml
Mancozeb (WP 800 g/kg)	Dithane	150 g
Kresoxim-methyl (WG 500 g/kg)	Stroby	10 g
Fenarimol (EC 120 g/l)	Rubigan	20 ml
Benomyl (WP 500 g/l)	Benlate	35 g
Myclobutanol (EC 125 g/l)	Systhane	15 ml
Iprodione (SC 500g/l)	Rovral Aquaflo	100 ml

Table 10.2. The corrected percentage mortality for adult *Aphelinus mali* females and mummies following exposure to various pesticides.

Chemical	Corrected Percentage mortality			
	24HRS	**48HRS**	**72HRS**	**MUMMIES**
Azinphos-methyl	100	100	100	14.70
Carbaryl(xlrplus)	100	100	100	9.04
Chlorpyrifos	100	100	100	0
Deltamethrin	100	100	100	5.17
Fenthion	96	100	100	3.57
Phosalone	100	100	100	1.73
Carbaryl(sevin)	100	100	100	0
Endosulfan	0	18.52	22.22	3.64
Flufenoxuron	0	0	0	---
Fenoxycarb	0	0	4.35	0
Methyl parathion	100	100	100	11.60
Vamidothion	100	100	100	0
Prothiophos	100	100	100	---
Chlorphenapyr	97.91	100	100	17.24
Penconazole	0	0	8.00	---
Captab	0	0	55.99	---
Copper oxychloride	0	0	63.998	---
Bupirimate	0	0	59.998	---
Mancozeb	0	0	3.996	---
Kresoxim-methyl	0	0	62.5	---
Fenarimol	0	23.08	31.997	---
Benomyl	0	7.99	74.99	---
Myclobutanol	0	0	0	---
Iprodione	0	0	0	---

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CHAPTER 11

CONCLUSIONS AND DISCUSSION

Although *Eriosoma lanigerum* (Hausmann) is a serious pest of apples in the Western Cape information on its development under South African conditions is limited and contradictory.

In order to develop a method for distinguishing between the developmental stages of *E. lanigerum* 13 characters were measured. The first instar can be distinguished by the absence of cornicles and the adult stage by the presence of the vulva. Body length and distance between the cornicles can be used to distinguish between instars 2, 3 and 4. However, an estimated 10% of the classifications into instars 2, 3 and 4 could be incorrect.

Although *E. lanigerum* developed over a wide range of temperatures in the laboratory, constant high temperatures of 30°C were detrimental as most nymphs died before completing their development. The net replacement rate (R_0) and intrinsic rate of increase (r_m) peaked at 20°C. This South African strain is well suited to temperatures between 20 and 23°C. The theoretical lower and upper threshold temperatures for development of *E. lanigerum* were estimated at 4.48 and 28.07°C respectively.

A definite upward migration of the first instar crawlers from the roots into the aerial parts of apple trees was recorded during spring. These crawlers started new *E. lanigerum* infestations in the aerial parts of the trees at the beginning of the summer. There was a correlation between the rainfall in spring and the number of crawlers moving up where trees were planted in soil that contained more clay than gravel. The clay soil remained sealed while still damp. Therefore, there were no cracks through which the crawlers could leave the roots for their upward migration. When the upward movement was delayed by rain, survival of the crawlers was negatively influenced by the high temperatures experienced later in the summer.

Usually *E. lanigerum* colonies became visible on the aerial parts of the trees from early summer and colonies parasitised by *Aphelinus mali* (Haldeman) were found from the middle of summer. At the end of summer colony numbers increased dramatically and during autumn, when the highest number of colonies was usually found, most of the colonies contained parasitised aphids and large numbers of black mummified aphids were visible. Large numbers of winged *E. lanigerum* appeared when colony numbers were high during February and March. The number of *E. lanigerum* colonies declined after April as a result of a reduced rate of reproduction due to cooler weather, high parasitism and the appearance of the winged aphids which do not reproduce. Although a few *A. mali* adults were recorded on the yellow traps during August and September their numbers stayed low until the end of summer when they began to increase. This was when most of the chemical sprays for the control of other insect pests ceased and higher temperatures favoured the development of the parasitoid in relation to *E. lanigerum.*

E. lanigerum overwintered on the tree and some *A. mali* adults were also active on warmer days in the orchard during winter. Rain and wind during winter

removed many of the colonies together with large numbers of mummified aphids containing diapausing *A. mali* larvae. At the end of winter many of the remaining aphids and diapausing parasitoids were removed by pruning and sprays applied to combat delayed foliation (DNOC and oil, winter oil), *Quadraspidiotus perniciosus* (Comstock) and *Pseudococcus* pests. Therefore, new infestations of the aerial parts during summer were initiated by the first instar (crawlers) migrating up from the roots during spring.

Although *A. mali* parasitised large numbers of *E. lanigerum* colonies, the parasitoid appeared too late in the season to prevent colony formation and bud damage. Corrective sprays using products like endosulfan and chlorpyrifos were often required. These products have a short residual action which makes the timing of sprays important. Therefore regular monitoring of population levels of *E. lanigerum* is necessary and a sampling system was developed. The unparasitised *E. lanigerum* colonies in leaf axils were counted on half of 25 trees per 2 ha. The colonies in wounds were omitted as they are not responsible for economic damage. An economic threshold of 5 colonies in leaf axils per half tree was set. At this threshold the sampling error was high (40 %) and resulted from the highly erratic infestation patterns of *E. lanigerum*. In a presence-absence sampling system, 5 infested leaf axils were equivalent to slightly more than 40% infested trees or 11 of the 25 trees per 2 ha infested. The reliability of decision making regarding the necessity for intervention was not seriously compromised using the presence-absence system instead of counting colonies and the time required for monitoring was reduced considerably.

In the Western Cape chemical products high in nitrogen, such as calcium nitrate, are applied for the control of bitterpit. Calcium nitrate is applied regularly from early to late summer when *E. lanigerum* crawlers are present in large numbers.

Calnitro® sprayed for the control of bitterpit stimulated the settlement of crawlers on Granny Smith trees but not on Royal Gala. Because it is difficult to control *E. lanigerum* colonies in the trees, reduction of initial colonisation would facilitate control. Therefore the use of chemicals for the control of bitterpit without nitrogen is recommended.

It was found that fruit weevil bands used for the control of *Phlyctinus callosus* Boh. limited the movement of *E. lanigerum* crawlers from the roots up into the trees but did not prevent colonisation. Where high number of crawlers migrated, bands became covered with crawlers and crawlers could walk over the sticky surface. Crawlers also disperse by wind, facilitating colonisation of the aerial parts. Although these bands only delayed colony formation, this can give *A. mali* time to recover from sprays and low spring temperatures, enhancing biological control. For the bands to be effective the trees must be free of *E. lanigerum* at the beginning of each season and the bands must be applied before the onset of the spring migration of crawlers from the roots up into the trees. The area under the tree must also be kept weed free so as not to supply additional means of entering the canopy of the apple tree.

The minimum threshold for development of the *A. mali* larval stage was 6.72°C with 172.41°D needed for development. High temperatures (above 27°C) did not adversely affect the development of the larval stage of *A. mali*, as was the case with *E. lanigerum*. However, the rate of development was slightly lower at 30°C than at 27°C. The minimum threshold temperature for pupal development was the same for males and females at 10.27°C with 109.89°D needed for pupal development. In this study parasitoids emerged earlier than those in the Netherlands (Evenhuis 1958) and Washington (Walker *et al.* 1988). This indicated that the local *A. mali* is better adapted to high temperatures than those from these countries. The high minimum

threshold temperature for development of *A. mali* in relation to *E. lanigerum* explained why there was an increase in aphid numbers during the cooler spring and autumn conditions.

During winter 50% of the mummified aphids contained diapausing *A. mali.* Diapausing *A. mali* collected during June emerged within 8 to 11 days at 25°C after being held in cold storage for between 12 and 17 weeks. This was the time needed to complete diapause development. Non-diapausing *A. mali* (pupae and adults) inside the mummies survived low temperatures (1-4°C) for up to 8 weeks and the developing larvae probably for 10-12 weeks. As there was no difference in the percentage emergence after cold storage from mummies collected during June, July or August it would be better to collect mummies early during winter when large numbers of mummies can be found in the trees. Parasitoids that completed diapause in cold storage showed no difference in the percentage that emerged at temperatures between 15 and 27°C. Mean (±SD) times to emergence ranged from 24.25±1.57 days at 15°C to 7.43±0.076 days at 27°C for males. Those for females ranged from 25.28±2.076 days at 15°C to 7.70±0.126 days at 27°C. The minimum threshold temperature for postdiapause development of *A. mali* was 10.15°C.

Branches with diapausing *A. mali* collected during early winter can be stored in a cool room and placed into the orchard during spring when diapause development has been completed. This can be done after the effect of detrimental sprays is no longer a threat to *A. mali* and after the upward movement of the *E. lanigerum* crawlers had started. This would augment the parasitoids that survived the winter and would ensure that the parasitoids have sufficient aphids to parasitise.

All the developmental stages of *E. lanigerum* were recorded from early summer until the end of winter. Winged females appeared from February until April,

when the number of individuals per colony was high. This together with parasitism by *A. mali* apparently resulted in a decline in the number of live aphids in each colony. Parasitism was recorded from the end of January until August. All the developmental stages of *E. lanigerum* were parasitised. However, the third, fourth and fifth instars (adults) were preferred. Although percentage parasitism reached high levels it was never 100% as many younger instars escaped parasitism while feeding under the larger aphids and mummified aphids. Superparasitism, with up to 9 larvae or eggs in one aphid, was recorded when parasitism was high. There was a correlation between the number of parasitised colonies in the tree and the number of parasitised individuals in a colony.

The effects of 14 insecticides, including 2 insect growth regulators, and 10 fungicides on the adult stage of the parasitoid, *A. mali*, were investigated under laboratory conditions. Some insecticides were also screened for their effect on the emergence of adult wasps from the mummified aphids. Most chemicals were highly toxic to the adult wasp within 24 hours, except endosulfan, the two growth regulators and the fungicides. The percentage emergence from the mummies was high for all chemicals tested. However, in the case of chlorpyrifos more than 60% of the adults died soon after emergence. Nearly 30% of the adults died soon after they emerged from mummies treated with carbaryl (XLR-Plus) and fenthion. The two insect growth regulators, flufenoxuron and fenoxycarb, did not adversely influence fecundity.

The chemicals that caused low or no mortality of the mummified aphids will be safer for use in IPM programs in apple orchards, as developing parasitoids within the mummy will escape the toxic effects of the chemicals, ensuring some survival of the parasitoids. The use of endosulfan as a corrective spray for *E. lanigerum,* growth

regulators for codling moth control and fungicides like penconazole, mancozeb, myclobutanol and iprodione against fungi should ensure better survival of *A. mali.*

The use of sprays to control *E. lanigerum* in the aerial parts of the trees can be kept to a minimum using the following management tactics:

1. Twigs infested with *E. lanigerum* can be removed during winter when normal pruning is done. This will reduce the overwintering population. Pruning wounds must be sealed as they provide refuges against wind and rain during winter.
2. During late winter or early spring the subterranean populations should be controlled to prevent the upward migration from the roots to the tree canopy. This can be done by using straw mulch (Damavandian 2000) or a soil application of imidacloprid (Pringle 1989).
3. During winter twigs bearing mummies containing *A. mali* can be collected from orchards and kept in cold storage. After upward migration has been completed these can be placed in orchards to augment the low numbers of *A. mali* surviving the winter. *E. lanigerum* crawlers appear on water shoots before they appear in the canopy of the trees. Therefore, to maximise survival of the wasps care should be taken to place the twigs bearing mummies in the vicinity of water shoots bearing colonies.
4. During summer *E. lanigerum* activity should be monitored at regular intervals (not longer than two weeks) by inspecting one half of 25 trees per 2 ha block. Corrective sprays should only be applied when 11 or more of the 25 trees have leaf axils infested with unparasitised colonies.
5. Chemicals which have the least detrimental effect on *A. mali* should be selected for the control of insect pests. Bioassay results indicated that the insect growth regulators flufenoxuron and fenoxycarb should be used for codling moth control

and endosulfan for corrective sprays against *E. lanigerum*. Stem bands can be used for the control of fruit weevil, *Phlyctinus callosus* Boh., as this will also retard colonisation of the tree canopy by *E. lanigerum*.

6. Sources of calcium used for bitterpit control which do not contain nitrogen should be identified, as these may not enhance the ability of crawlers to settle as is the case with calcium nitrate which is currently used.
7. Plant material used for establishing new orchards should be free of *E. lanigerum*.

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